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THE UNIVERSITY OF ALBERTA

SYNTHETIC APPROACHES TO AGROCIN 84 AND RELATED NUCLEOSIDE ANALOGUES

BY

© RAVI S. VINAYAK

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA
FALL, 1986

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SYNTHETIC APPROACHES TO AGROCIN 84
AND RELATED NUCLEOSIDE ANALOGUES

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To

My Parents and Teachers

ABSTRACT

This dissertation reports studies directed toward the synthesis of agrocin 84, a highly selective nucleotide antibiotic effective against crown gall plant disease. Syntheses of the three major molecular components and/or analogues were achieved. Attempted couplings of the components to give the antibiotic were not successful.

The "core nucleoside" of agrocin 84 was prepared by three routes. Conversion of 9-(β-*D*-arabinofuranosyl)-adenine into 9-(2,3-anhydro-β-*D*-lyxofuranosyl)adenine followed by reduction of this nucleoside epoxide with sodium borohydride in ethanol gave 9-(3-deoxy-β-*D*-*threo*-pentofuranosyl)adenine in high yields. A second approach utilized selective treatment of adenosine with 2,4,6-triisopropylbenzenesulfonyl chloride to give 2'-*O*- and 3'-*O*-(2,4,6-triisopropylbenzenesulfonyl)adenosines followed by a reductive [1,2]-hydride shift rearrangement with lithium triethylborohydride to give 9-(3-deoxy-β-*D*-*threo*-pentofuranosyl)adenine and 9-(2-deoxy-β-*D*-*threo*-pentofuranosyl)adenine in a ratio of 1:2. In the third approach, 9-(β-*D*-arabinofuranosyl)adenine was treated with tert-butyldimethylsilyl chloride to give a mixture of 2',5'- and 3',5'-di-*O*-tert-butyldimethylsilylarabinosyladenines. Functionalization of the free hydroxyl group of

these protected nucleosides with phenyl chlorothionocarbonate followed by deoxygenation with tri-*n*-butyl stannane and cleavage of the silyl ethers with tetra-*n*-butylammonium fluoride gave 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine as the major product.

Selective hydrolysis of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose gave 1,2-*O*-isopropylidene-α-D-glucofuranose which was tribenzylated. Treatment of this product with hydrogen chloride in methanol followed by benzylation of the free 2-hydroxyl group gave methyl 2,3,5,6-tetra-*O*-benzyl-D-glucofuranoside. Hydrolysis of this glycoside with 60% acetic acid gave 2,3,5,6-tetra-*O*-benzyl-D-glucofuranose with an α:β ratio of 3:2.

A modified Knoevenagel condensation of isobutyraldehyde with ethyl hydrogen malonate gave ethyl (*E*)-4-methylpent-2-enoate. Dihydroxylation of the latter compound with osmium tetroxide followed by ammonolysis gave DL-*threo*-2,3-dihydroxy-4-methylpentanamide.

Model studies on the syntheses of 6-*N*-(phosphoryl)-adenosine nucleosides were pursued to evaluate conditions for attempted coupling of the glucofuranosyl moiety to the 6-amino function of the "core nucleoside" via a glycosylphosphoramidate linkage. Treatment of 2',3',5'-tri-*O*-acetyladenosine with phenyl phosphorodichloridate followed by reaction with various alcohols in the presence of silver carbonate and deprotection of acetyl groups gave a number of 6-*N*-(phosphoryl)adenosine nucleosides.

In an alternate approach, a phosphite-azide coupling method with 2',3',5'-tri-*O*-acetyl-6-azido-9-β-D-ribofuranosylpurine was examined for construction of the glycosylphosphoramidate linkage. This was successful for the synthesis of 6-*N*-[ethyl[illegible]yl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosid-6-yl)phosphoryl]adenosine. However, attempts to couple several sugars through the anomeric hydroxyl group did not prove successful.

Model studies were also directed towards syntheses of nucleosides containing an R-C(O)-NH-P(O)- function at the 5'-hydroxyl group in order to evaluate conditions for attachment of DL-*threo*-2,3-dihydroxy-4-methylpentanamide to the 5'-hydroxyl function of the "core nucleoside" via a phosphoramidate linkage. *N*-Octanoyl-di-*O*-ethylphosphoramidate was prepared by acid hydrolysis of *N*-octanoyl-tri-*O*-ethylphosphorimidate which in turn was obtained by reaction of triethyl phosphite with octanoyl azide. These studies resulted in the synthesis of 6-*N*-benzoyl-5'-*O*-[(*N*-benzoyl)phosphoramidatyl]-2',3'-*O*-isopropylideneadenosine.

A four stage synthesis of diethyl (2,3,5,6-tetra-*O*-benzyl-α-D-glucofuranosyl)methylphosphonate from 2,3,5,6-tetra-*O*-benzyl-D-glucofuranose was performed. A direct approach to the synthesis of diethyl (2,3,5,6-tetra-*O*-

benzyl-D-glucofuranosyl)methylphosphonate with a α:β ratio of 3:2 involved treatment of 2,3,5,6-tetra-*O*-benzyl-D-glucofuranose with tetraethyl methylenebisphosphonate and sodium hydride.

Finally, attempts were made to synthesize compounds with carbonyl functions analogous to the phosphoryl group at O5' in agrocin 84. There resulted in the syntheses of 6-*N*-benzoyl-5'-*O*-[(*N*-octanoyl)carbamoyl]-9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine, 6-*N*-benzoyl-5'-*O*-[(*N*-octanoyl)carbamoyl)adenosine and 5'-deoxy-5'-*N*-[(*N*-octanoyl)carbamoyl]amino-6-*N*-formyladenosine.

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TABLE OF CONTENTS

Page

ABSTRACT..v
ACKNOWLEDGEMENTS..ix
LIST OF TABLES...xii
LIST OF FIGURES...xiii
LIST OF SCHEMES...xiv
ABBREVIATIONS...xviii

INTRODUCTION

A. Crown gall disease 1
B. Control of crown gall disease..........................5
C. Agrocin 84...11

RESULTS AND DISCUSSION

A. Syntheses of 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine and 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine...............23
B. Synthesis of 2,3,4,6-tetra-*O*-benzyl-D-glucofuranose.................................36
C. Synthesis of DL-*threo*-2,3-dihydroxy-4-methylpentanamide...........................40
D. Nomenclature for 6-*N*-(phosphoryl)adenosines....43
E. Syntheses of 6-*N*-(phosphoryl)adenosine derivatives...................................46
F. UV spectral behaviour of 6-*N*-(phosphoryl)-adenosine nucleosides..........................68

Table of Contents (continued):

Page

G. Fast atom bombardment mass spectrometry........71
H. Studies directed toward nucleoside-5'-phosphoramidates..............................73
I. Syntheses of sugar phosphonates................83
J. Syntheses of compounds analogous to agrocin 84......................................91

EXPERIMENTAL..95

BIBLIOGRAPHY...155

LIST OF TABLES

Page

1. UV spectral behaviour of 2',3',5'-tri-O-acetyl-6-N-(phosphoryl)adenosine nucleosides....69

2. UV spectral behaviour of 6-N-(phosphoryl)-adenosine nucleosides....69

LIST OF FIGURES

Page

1. Effect of agrocin 84 on protein, DNA and RNA synthesis in Agrobacterium tumefaciens H-38-9....................................7

2. UV spectrum for agrocin 84...........................15

LIST OF SCHEMES

Page

I Degradation products of agrocin 84............18

II Synthesis of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (17) and 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3) by Goodman *et al*..............................24

III Synthesis of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (17) by Ikehara *et al*........26

IV Synthesis of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (17) and 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3) by Mengel and Wiedner..........................27

V Synthesis of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (17) and 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3) by Hansske and Robins..............................28

VI Synthesis of 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3) from 9-β-D-(arabinofuranosyl)adenine (32)........................30

VII Possible mechanism for the formation of 9-(2,3-anhydro-β-D-lyxofuranosyl)-adenine (27)..................................30

VIII [1,2]-Hydride shift rearrangement of 2'-*O*- and 3'-*O*-TPS-adenosines (36) to give 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3) and 9-(2-deoxy-β-D-*threo*-pentofuranosyl)-adenine (17)..................................32

IX Application of the Robins-Wilson deoxygenation procedure to prepare 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3)...............34

List of Schemes (continued):

Page

X Synthesis of 2,3,5,6-tetra-O-benzyl-D-glucofuranose (47)....38

XI Synthesis of DL-threo-2,3-dihydroxy-4-methylpentanamide (7')....42

XII Syntheses of 6-N-(phosphoryl)adenosine nucleosides by Charubala and Pfleiderer....48

XIII Syntheses of 6-N-(phosphoryl)adenosine nucleosides by the phenyl phosphorodichloridate approach....52

XIV Suggested mechanism for the formation of 2',3',5'-tri-O-acetyl-6-N-(diphenylphosphoryl)adenosine (58)....54

XV Suggested mechanism for the formation of 2',3',5'-tri-O-acetyl-6-N-(phosphoryl)-adenosine nucleosides....54

XVI Hydrogenolysis of 2',3',5'-tri-O-acetyl-6-N-(benzylphenylphosphoryl)adenosine (80a) and 6-N-(benzylphenylphosphoryl)-adenosine (81a)....55

XVII Formation of 6-N-(phosphoryl)adenosines by reaction of 2',3',5'-tri-O-acetyl-6-azido-9-β-D-ribofuranosylpurine (91) with triethyl and triphenyl phosphite....58

XVIII Attempted syntheses of 6-N-(glucosylphosphoryl)adenosines by reaction of 2,3,4,6-tetra-O-benzyl-α-D-glucopyranose (95) with alkyl and aryl phosphorochloridites and phosphorodichloridites....60

List of Schemes (continued):

Page

XIX Synthesis of 6-*N*-[ethyl(methyl 2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosid-6-yl)phosphoryl]adenosine (101)....62

XX Possible mechanisms for the formation of nucleoside phosphoroamidates....63

XXI Synthesis of 5'-deoxy-5'-*N*-(diethylphosphoryl)amino-2',3'-*O*-isopropylideneadenosine (113) and 5'-deoxy-5'-*N*-(diphenylphosphoryl)-amino-2',3'-*O*-isopropylideneadenosine (114)....64

XXII Attempted syntheses of 6-*N*-(glucosylphosphoryl)adenosines from phosphorus (III) intermediates....67

XXIII Synthesis of *N*-octanoyl-di-*O*-ethylphosphoramidate (145) and 5'-*O*-(phenylphosphoramidatyl)-2',3'-*O*-isopropylideneadenosine (148)....78

XXIV Synthesis of 6-*N*-benzoyl-5'-*O*-[(*N*-benzoyl)-phosphoramidatyl]-2',3'-*O*-isopropylidene-adenosine (152)....79

XXV Synthesis of bis(6-*N*-benzoyl-2',3'-*O*-isopropylideneadenosin-5'-yl) 2-chlorophenyl phosphate (156)....81

XXVI Synthesis of diethyl (161a) and dimethyl (2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranosyl)-methylphosphonate (161b)....84

XXVII Synthesis of diethyl (2,3,5,6-tetra-*O*-benzyl-α-*D*-glucofuranosyl)methylphosphonate (165)....86

XXVIII Synthesis of diethyl (2,3,5,6-tetra-*O*-benzyl-α- and β-*D*-glucofuranosyl)methylphosphonates (165 and 166)....88

List of Schemes (continued):

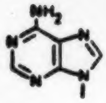
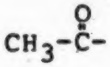
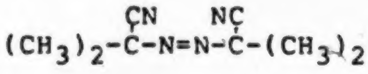
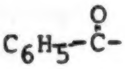
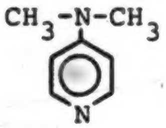
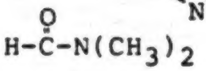
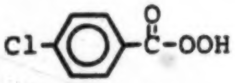
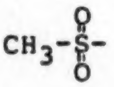
Page

XXIX Synthesis of (2,3,5,6-tetra-O-benzyl-α and β-D-glucofuranosyl)methyl alcohols (172 and 173)....................................90

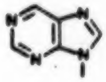
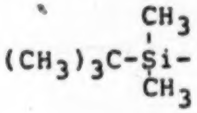
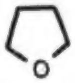
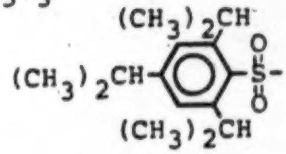
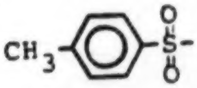
XXX Synthesis of 6-N-benzoyl-5'-O-[(N-octanoyl)-carbamoyl]adenosine (177) and 6-N-benzoyl-5'-O-[(N-octanoyl)carbamoyl]-9-(3-deoxy-β-D-threo-pentofuranosyl)adenine (179).........93

XXXI Synthesis of 5'-deoxy-5'-N-[(N-octanoyl)-carbamoyl]-6-N-formyladenosine (183)...........94

ABBREVIATIONS USED

A	Adenin-9-yl	
Ac-	Acetyl	 $CH_3-\overset{O}{C}-$
AIBN	Azobisisobutyronitrile	 $(CH_3)_2-\overset{CN}{C}-N=N-\overset{NC}{C}-(CH_3)_2$
Bn-	Benzyl	$C_6H_5-CH_2-$
t-Bu-	*t*-Butyl	$(CH_3)_3C-$
Bz-	Benzoyl	 $C_6H_5-\overset{O}{C}-$
DEAD	Diethyl azodicarboxylate	$C_2H_5O_2C-N=N-CO_2C_2H_5$
DMAP	4-Dimethylaminopyridine	
DMF	*N,N*-Dimethyl formamide	 $H-\overset{O}{C}-N(CH_3)_2$
DMSO	Dimethylsulfoxide	$(CH_3)_2S=O$
Et-	Ethyl	CH_3CH_2-
HMPA	Hexamethylphosphoramide	$[(CH_3)_2N]_3P=O$
LTBH	Lithium triethylborohydride	$Li(C_2H_5)_3BH$
MCPBA	m-Chloroperoxybenzoic acid	
Me-	Methyl	CH_3-
Ms-	Methanesulfonyl	 $CH_3-\overset{O}{\underset{O}{S}}-$

Abbreviations (continued):

Ph-	Phenyl	C_6H_5-
PLC	Preparative layer chromatography	
i-Pr	Isopropyl	$(CH_3)_2CH$-
Pu	Purin-9-yl	
TBDMS-	*t*-Butyldimethylsilyl	 $(CH_3)_3C-\overset{CH_3}{\underset{CH_3}{Si}}-$
TBHP	*t*-Butyl hydroperoxide	$(CH_3)_3COOH$
THF	Tetrahydrofuran	
TLC	Thin layer chromatography	
TPP	Triphenylphosphine	$(C_6H_5)_3P$
TPS-	2,4,6-Triisopropylbenzene-sulfonyl	
Ts-	p-Toluenesulfonyl-	

INTRODUCTION

A. Crown Gall Disease

Crown gall[1-6] is a neoplastic malignancy (plant tumor) initiated by a gram negative bacterium, Agrobacterium tumefaciens, which occurs on an extremely broad range of host plants. It was first assigned a bacterial aetiology by Smith and Townsend.[7] Parallelism between crown gall and animal tumors was highlighted by the observation of White and Braun[8] who proved crown gall to be a true oncogenic transformation. They[8] also showed that crown gall tissue cultured in the absence of the bacterium can be maintained indefinitely in axenic culture without the loss of its tumorous characteristics.[9] Although the disease occurs in a remarkably wide variety of higher plants, it can only be induced in freshly wounded tissue.[10] Oncogenic strains of the bacterium contain a large plasmid,* part of which is transferred to the plant cell during the oncogenic transformation. It is maintained in the transformed cell indefinitely[11] and in this respect crown gall resembles the virally induced animal tumors. Transformed tissue has the ability to form an overgrowth when grafted onto a healthy plant, has capacity for growth in tissue culture without added plant hormones[12] and can synthesize 'opines'[13,14] (unusual derivatives of basic amino acids not found in normal tissue which are specifically synthesized by crown gall plant cells and used by the inhibiting agrobacteria as specific growth substances). The study of crown gall may help in the understanding of carcinogenesis and should be important in elucidating the control of gene expression in higher plants. The feasibility of using agrobacterium plasmids as vehicles for genetic engineering in higher plants is also of interest.

---

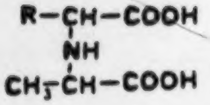
* Plasmids are autonomously replicating closed-loop DNA molecules that are independent of the bacterial DNA and that can exist free in the cytoplasm of a bacterium; they can be transmitted only by cell division or cell to cell contact, but cannot be integrated into chromosomes of the host.

Significant achievements have been made only recently on the nature of the agent that causes the induction of crown gall tumors. This is primarily because studies were directed at the causal organism rather than at the host plant. Moreover, techniques have been developed in molecular biology that are directly applicable to studies of the elusive tumorigenic substance known as the tumor inducing principle (TIP).[15,16]

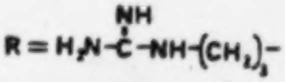
Not all agrobacterium plasmids confer the ability to induce tumors. Those that do are designated tumor inducing (Ti) plasmids[17,18] and they promote bacterial conjugation.[19-21] The genes required for their transfer are closely associated with opine metabolism and are activated by the characteristic opine.[22,23] There is some evidence that opines enhance tumor growth.[24,25]

Structural formulae of opines synthesized by crown gall tumors are given below:

Octopine family



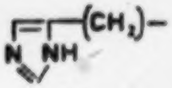
$$\begin{array}{l} R-CH-COOH \\ \quad\;\; | \\ \quad\;\; NH \\ \quad\;\; | \\ CH_3-CH-COOH \end{array}$$

Octopine: 

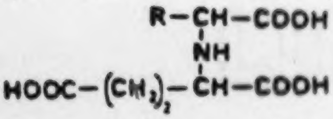
$R=H_2N-\overset{NH}{\overset{\|}{C}}-NH-(CH_2)_3-$

Octopinic acid: $R=H_2N-(CH_2)_3-$

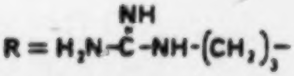
Lysopine: $R=H_2N-(CH_2)_4-$

Histopine: R= 

Nopaline family



$$\begin{array}{r} R-CH-COOH \\ | \quad\quad\;\; \\ NH \quad\quad \\ | \quad\quad\;\; \\ HOOC-(CH_2)_2-CH-COOH \end{array}$$

Nopaline: 

$R=H_2N-\overset{NH}{\overset{\|}{C}}-NH-(CH_2)_3-$

Nopalinic acid or Ornaline: $R=H_2N-(CH_2)_3-$

Tumor induction by agrobacteria is a largely unknown process. It is controlled by agrobacterial plasmid DNA and also, possibly, by chromosomal genes of the bacteria. During the tumor induction, the bacteria enter the intracellular spaces of wound sites on plants. The injured cells multiply and then become attached to specific sites on normal cell walls.[26] Agrobacteria do not enter the healthy plant cells, but transform them into tumor cells probably by injecting a piece of DNA into them. These sequences referred to as T-DNA are maintained[11] and transcribed[27-29] in the transformed plant cells. The T-DNA is essential for maintenance of tumorous state[30,31] and for the biosynthesis[31] of the opines.

In terms of new concepts in the biology of host-parasite reactions, the study of crown gall induction has been rewarding. It has shown that certain bacteria have the capacity to introduce specific genetic information in many different plant hosts. Also, the transformed plant cells produce opines, which are of ecological value to the bacteria. This is the first example of a natural mechanism of transfer of genetic information from prokaryotes to eukaryotes. Furthermore, the crown gall phenomenon illustrates a new kind of plant parasitism and/or symbiosis in which one of the partners (parasite, in this case) not only adopts the properties of the other (host) but also changes 'its' genetic properties. Schell et al.[32] have termed this type of interaction as "genetic colonization".

B. Control of Crown Gall Disease

Successful biological control of crown gall with agrobacterium radiobacter 84, which produces the active factor agrocin 84,[33] has been reported.[34-45] This represents the first commercial use of a specific microorganism to control plant pathogens in soil and of a bacterium to control any plant disease.

Agrocin 84 is a general inhibitor of pathogenic strains of Agrobacterium of the same or related species. Nearly all non-pathogens are unaffected. This type of inhibition is similar to that of colicins.[46] Growth inhibition by agrocin 84 has been correlated with pathogenicity[33,47-51] and the presence of a nopaline tumor inducing plasmid.[48-50] This correlation results from the fact that both the agrocin 84 sensitivity and tumor inducing ability are properties controlled by DNA sequences located on a large plasmid (the Ti-plasmid) present in all A. tumefaciens strains.

This conclusion[48] was reached by demonstrating:

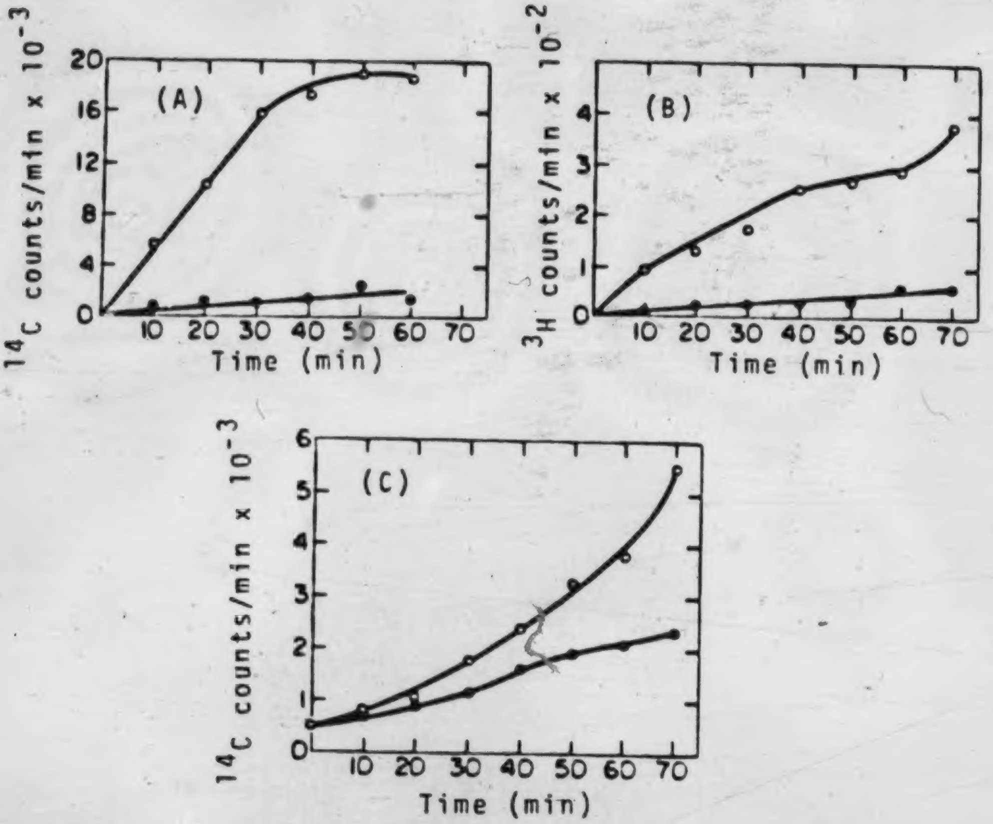
1. that an oncogenic agrocin 84 sensitive strain C58 when cured* of its Ti-plasmid becomes resistant to the action of agrocin 84.

---

* Resulting in plasmid-free cells.

2. that when agrocin 84 resistant C58 colonies are selected they invariably have lost both their tumor inducing ability and the Ti-plasmid simultaneously.
3. that the kinetics of appearance of Ti-plasmid cured cells are identical to those of agrocin 84 resistant cells, as a result of growth at 37°C of C58 strain.

The agrocin 84 produced by A. radiobacter K84 interacts[52,53] specifically with the cell wall of the virulent strain of A. tumefaciens H-38-9 and does not adsorb onto the surface of the avirulent strain that was originally derived from the same parent. The bacterial cell surface is an important factor in virulence as has been suggested by Lippincott and Lippincott.[26] They postulate a specific receptor on the bacterial surface that interacts with a specific site on the plant cell wall to initiate crown gall disease. Protein synthesis, as measured by incorporation of ^{14}C labeled amino acids, was almost completely inhibited within ten minutes by addition of agrocin 84 (Fig. 1A). A similar pattern of inhibition occurred in the DNA synthesis, as measured by the incorporation of [^3H]thymidine (Fig. 1B). The inhibition of DNA synthesis was accompanied by degradation of DNA as is the case with both colicin E2[54] and megacin C.[55] The inhibitory effect on RNA synthesis was not apparent until twenty minutes (Fig. 1C) after the addition of agrocin 84



Effect of agrocin B4 on synthesis
in *A. tumefaciens* H-38-9.

Fig.1. (A) Protein synthesis
at zero time. ^{14}C-labeled
mixed amino acids were added.
(B) DNA synthesis at zero time.
^{3}H-thymidine was added.
(C) RNA synthesis at zero time.
^{14}C-uracil was added.
Symbols: ●,agrocin B4; o,control.

and resulted in a 50% reduction of incorporation of [^{14}C]uracil when compared to the control cells. The transport of amino acids across the cell membrane was immediately stopped by the addition of agrocin 84 demonstrating an effect on the integrity of the cell membrane and associated transport functions.

According to recent studies by Murphy and Roberts,[56] agrocin 84 is transported inside sensitive strain K57A possibly by means of a transport protein found in its periplasmic space. The unique selectivity of agrocin 84 possibly resides in the presence of a specific binding protein in the sensitive strain K57A, which is released into the surrounding medium in the presence of carbenicillin and is presumably periplasmic in origin. They[56] suggest that agrocin 84 is the first recorded member of a new class of highly selective low molecular weight nucleotide bacteriocins. (Bacteriocins have been defined as non-replicating, bactericidal substances apparently protein in nature which are produced by certain strains of bacteria and are active against some other strains of the same or closely related species.[46])

Studies by Sule and Kado[57] show that a predominant number of agrocin 84 resistant mutants of A. tumefaciens C58 and O are virulent and harbor nopaline Ti plasmids. The mechanism by which these agrocin-resistant mutants operate is uncertain. Their studies clearly show high levels of $[^{32}\text{P}]$agrocin 84 uptake by sensitive strains and their respective sensitive mutants. However, agrocin 84 resistant mutants and transconjugants harboring Ti plasmids from these mutants display low levels of agrocin 84 uptake ability, thereby suggesting that impairment of the agrocin uptake mechanism results in resistance to agrocin 84.

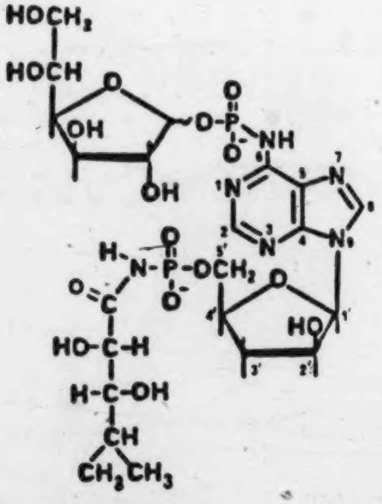
To ascertain mechanisms that are operating in crown gall cells and also to probe the mode of action of agrocin 84 in relation to crown gall-inducing potency, Das *et al*.[58] have studied the isoenzyme pattern of some hydrolytic enzymes viz. alkaline phosphatase, acid phosphatase and catalase in the virulent and avirulent strains of *A. tumefaciens* using polyacrylamide gel electrophoresis. Their studies suggested that agrocin 84 resistance was accompanied by genetic repression in regard to synthesis of one of the isoenzymes in each of these three enzymes. Avirulent resistant strains also showed a reduction in the activities of all hydrolases.

Studies have also been carried out in detail on the lipid make up of *A. tumefaciens* using virulent agrocin-sensitive and avirulent agrocin-resistant strains[59] and also on the biochemical characteristics of cytoplasmic membranes of various strains of *A. tumefaciens*.[60] It has been shown that phosphatidylcholine, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, and phosphatidylglycerol are the major phospholipids present in A. tumefaciens. Although, the total lipid content is increased due to development of resistance towards agrocin 84, the proportion of phospholipids and neutral lipids in the total lipid fraction remains almost the same either in the normal or in the resistant strains. Also, agrocin resistance brought about an increase in the amount of unsaturated fatty acid content in A. tumefaciens as obtained by gas-liquid chromatographic analysis. No appreciable differences were detected in the phospholipids, neutral lipids, and fatty acid components of the whole cell and cytoplasmic membrane or among the cytoplasmic membrane fragments of the three strains studied viz. A. tumefaciens 14 (agrocin sensitive), A. tumefaciens 14 R (agrocin resistant), and a plasmid containing resistant strain A. tumefaciens S 1005.

The plasmid responsible for coding agrocin 84 has been genetically isolated and characterized.[61] The addition of a genetically selectable trait to this plasmid and experiments which show that it is in itself a conjugative plasmid have been carried out by Farrand *et al.*[62] They[62] also present a functional map of pAgk 84 locating regions in agrocin production and conjugative transfer.

C. Agrocin-84

Agrocin 84 (1), an extraordinarily selective antibiotic produced by Agrobacterium radiobacter K84 is an adenine nucleotide analog[63-65] that inhibits nucleic acid[52,53,65] and protein synthesis[53,65] and blocks the attachment of virulent A. tumefaciens cells to its host cells.[66] It is considered bacteriostatic[66] and inhibits growth.[33,52] Sensitivity of A. tumefaciens to agrocin is conferred by genes on the Ti plasmid[48] which also govern virulence,[49,67,68] nopaline metabolism,[69,70] and host specificity.[71-73] The accepted purine and sugar numbering system is illustrated in the structure of agrocin 84 (1).



(1)

a) Isolation

In 1960, Stonier[74] first observed that certain strains of Agrobacterium tumefaciens produced bacteriocins which had unusually low molecular weights and were resistant to proteolytic enzymes. Kerr and Htay[33] demonstrated that a similar bacteriocin, known as agrocin 84, selectively inhibited growth of most pathgenic strains of A. tumefaciens, although the growth of most non-pathogenic strains was unaffected.

Agrocin 84 is produced by culturing a rough colony variant strain of A. radiobacter var. radiobacter in a defined liquid medium.[63] After preliminary work,[53] based on a gel filteration of crude agrocin 84 and amino acid analysis of effluent peaks McCardell and Pootjes[53] erroneously suggested agrocin 84 to be a small peptide with a molecular weight of 2,500 containing six different amino acids, including nine molecules of glutamine or glutamic acid and seven molecules of serine. Schell et al.[75] who had earlier outlined a partial purification procedure for agrocin 84, indicated it to be an acidic substance of low molecular weight. Kerr *et al.*[63] have reported a 10,000-fold purification of agrocin 84 by gradient elution techniques involving charcoal adsorption and anion exchange chromatography on DEAE sephadex. Final purification was achieved by electrophoresis on glass fibre filter paper. Pootjes *et al.*[65] have published a procedure for the rapid purification of milligram quantities of agrocin 84 from the high yield strain *Agrobacterium radiobacter*, K84. This procedure, which employed charcoal adsorption, ion-exchange and sieving chromatography, and continuous-flow electrophoresis yielded agrocin 84 which was 65% pure on a dry weight basis.

b) Structure of Agrocin 84[63-65]

Agrocin 84 (1) has been partially characterized as a disubstituted fraudulent nucleotide, with a 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine nucleoside core (3). The reported structural evidence shows the presence of a 5'-phosphoryl linkage from the nucleoside core to the amide group nitrogen of D-*threo*-2,3-dihydroxy-4-methylpentanamide (7) and also a D-glucofuranosyloxyphosphoryl substituent at N6 of adenine.

The evidence leading to the structure of agrocin 84 is as follows:

The IR spectrum of agrocin 84 showed the presence of a P-O stretching frequency at 1225 cm^{-1} and the absence of simple carbonyl and pyrophosphate groupings.

The UV spectrum of agrocin 84 (Fig. 2) at neutral pH had a maximum absorbance at 264 nm, a shoulder at 273 nm and a minimum at 227 nm. Upon acidification (pH 1.1) the absorbance peak shifted to 268 nm. No spectral changes occurred when samples were made alkaline. These properties are characteristic of 6-N-acylated 9-substituted adenine nucleosides.[76,77] Hydrolysis of agrocin 84 at pH 1.1 for 18 hours resulted in a compound with altered UV absorbance characteristics which were similar to those of a 9-substituted adenine derivative ($\lambda_{max}^{pH\ 7.0}$ = 259 nm, $\lambda_{max}^{pH\ 1.1}$ = 257 nm).[78] The reported bathochromic shift for 6-N-acylated 9-substituted adenine nucleoside derivatives at pH ~ 1 and the known thermolability and acid lability of phosphoramidates led to postulate

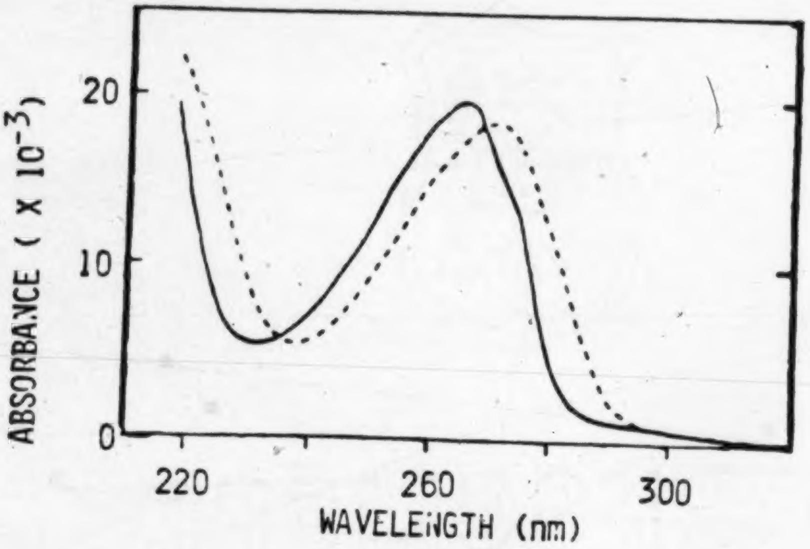


FIG. 2

UV SPECTRUM FOR AGROCIN 84
at pH 1 (----) and
at pH 7 (——).

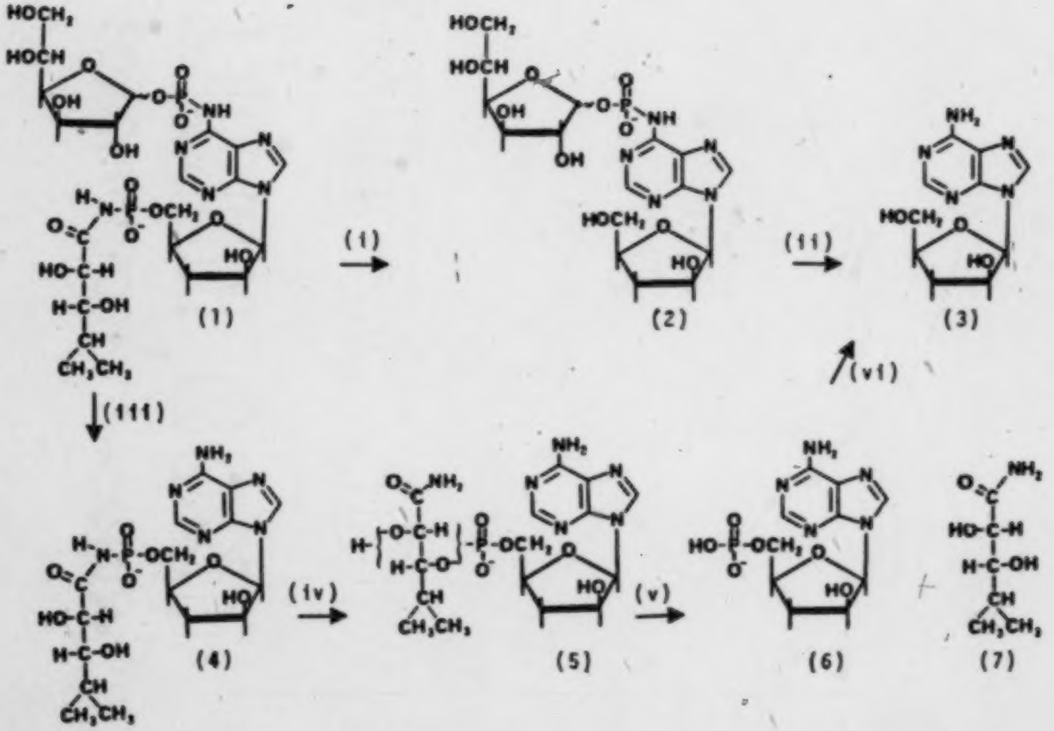
the presence of a 6-N-phosphoramidate grouping. Furthermore, 6-N-(cyanoethylphosphoryl)-adenosine[63] and agrocin 84 had the same UV spectral characteristics: $\lambda_{max}^{pH=1} = 264$ nm, $\lambda_{max}^{pH=13} = 267.5$ nm.

Mild alkaline hydrolysis (1 M NH_4OH, 25°C, 24 h) of agrocin 84 (1) produced nucleotide (2) containing D-glucose with the characteristic absorption maximum (264 nm, ϵ 19,500) of an adenine nucleoside-6-N-phosphoramidate (Scheme I). In addition, the non-UV absorbing organic phosphate monoesters from a postulated initial cyclic product yielded inorganic phosphate and a species containing a vicinal glycol after dephosphorylation with Escherichia Coli alkaline phosphatase. This glycol was shown to be D-threo-2,3-dihydroxy-4-methylpentanamide (7) by the usual physical and spectral criteria.

Periodate oxidation of agrocin 84 yielded one mole each of formaldehyde and isobutyraldehyde, whereas similar treatment of nucleotide (2) yielded only one mole of formaldehyde. As no glycol containing substituents other than glucose were detectable in nucleotide (2) after further hydrolysis to the nucleoside (3) and inorganic phosphate, it follows that glucose was present as a glucofuranosyloxyphosphoramidate and that neither the 2-, nor the 3-, hydroxyl of D-threo-2,3-dihydroxy-4-methylpentanamide (7) was esterified to phosphate in agrocin 84. The configuration of the anomeric glucofuranosyl linkage remains uncertain. Further alkaline hydrolysis of nucleotide (2) yielded the nucleoside 9-(3-deoxy-β-D-threo-pentofuranosyl)adenine (3) and a postulated cyclic gluco-furanosyl phosphate. The structure of the 'fraudulent' core nucleoside (3) was determined by chromatographic and electrophoretic comparison with an authentic sample synthesized by Goodman et al.[79]

Brief heating of an aqueous solution of agrocin 84 (100°C, pH 7, 15 mins) produced nucleotides (4) and (5), which exhibited absorption maxima at 260 nm, plus a postulated cyclic glucofuranosyl phosphate. Nucleotide (4) formed a borate complex, underwent periodate oxidation to yield one mole of isobutyraldehyde and was slowly cleaved by snake venom phosphodiesterase to produce nucleoside phosphate monoester (6). Nucleotide (4)

SCHEME I



(i) 1M NH_4OH, 25°C, 24h; (ii) 1M NH_4OH, 110°C, 1h; (iii) 110°C, pH 7, 15min; (iv) 0.1M HCl, 25°C, 17h; (v) snake venom phosphodiesterase, 25°C, pH 8.8, 3h; (vi) snake venom 5'-nucleotidase, 25°C, pH 9, 1h.

irreversibly isomerized to the periodate insensitive, non-borate complexing nucleotide (5) by dilute acid treatment. Nucleotide (5) was readily cleaved by snake venom phosphodiesterase to produce (6) and one mole of amide (7). Finally, the nucleoside phosphate monoester (6) was readily cleaved by a specific snake venom 5'-nucleotidase to nucleoside (3). The latter observation combined with the formation of isobutyraldehyde from nucleotide (4) and agrocin 84 (1) suggested the presence of a second phosphoramidate linkage in agrocin 84, from the 5'-hydroxyl of the nucleoside to the amide nitrogen atom, but the possibility that the linkage to the amide group by way of the enolic form to the oxygen atom is not excluded.

The calculated molecular weight of 904.4 for the anhydrous bis-triethylammonium salt $C_{34}H_{66}N_{8}O_{16}P_{2}$ was in accord with the predicted[63] maximum value of 1,100 ± 100. This was set by an $A^{1\%}_{1cm} = 180$ and an ϵ value of 19,860 at 264 nm and neutral pH for the purest samples obtained.

c) Structure-activity relationship

Murphy *et al*.[80] have published a structure-function study of agrocin 84. Two agrocin 84 nucleotide fragments (2 and 4) lacking either the N6 (4) or 5'-phosphoramidate (2) substituents were used in uptake studies of [$^{32}P_2$]-agrocin 84.

The remarkable bacteriocin-like specificity of agrocin 84 appears to be due to its uptake into strains containing a plasmid-coded periplasmic protein, which recognizes and binds to complementary sites on the agrocin 84 molecule.[56] The study indicated the absolute requirement for the 6-*N*-D-glucofuranosyloxyphosphoramidate substituent of agrocin 84 for this specific uptake, whereas the 5'-phosphoramidate grouping was of little importance. The competitive inhibition of the uptake of agrocin 84 by structure (2) but not by structure (4) pointed to the primary role of the N6 substituent in the binding-recognition step of agrocin 84 transport.

Structure (4) exhibited biological activity against both K57A and K57 strains. The presumed uptake by diffusion or a low affinity transport system would be independent of Ti plasmid control. Since agrocin 84 was totally inactive against the K57 strain, it was suggested that either the N6 substituent impeded transport into agrobacteria (unless the specific Ti plasmid-coded agrocin 84 binding protein was present) or that agrocin 84 entered the non-pathogenic strains in a manner similar to structure (4) and the enzymes required for further modification to a toxic metabolite were absent.

The important feature established by this study was that the uptake of a molecule containing a 'fraudulent' nucleoside moiety (3) was not sufficient for growth inhibition of the bacteria. Thus, structure (2) was readily taken up by the pathogenic strain K57A, but this compound was shown to be non-toxic.[64] On the other hand, the barely measurable uptake of structure (4) was sufficient to cause marked inhibition of growth.[64] The presence of a 5'-phosphoryl substituted nucleoside was therefore necessary for toxicity. The loss of activity by conversion of structure (4) to (5) showed that toxicity may depend on a 5'-phophoramidate bond.

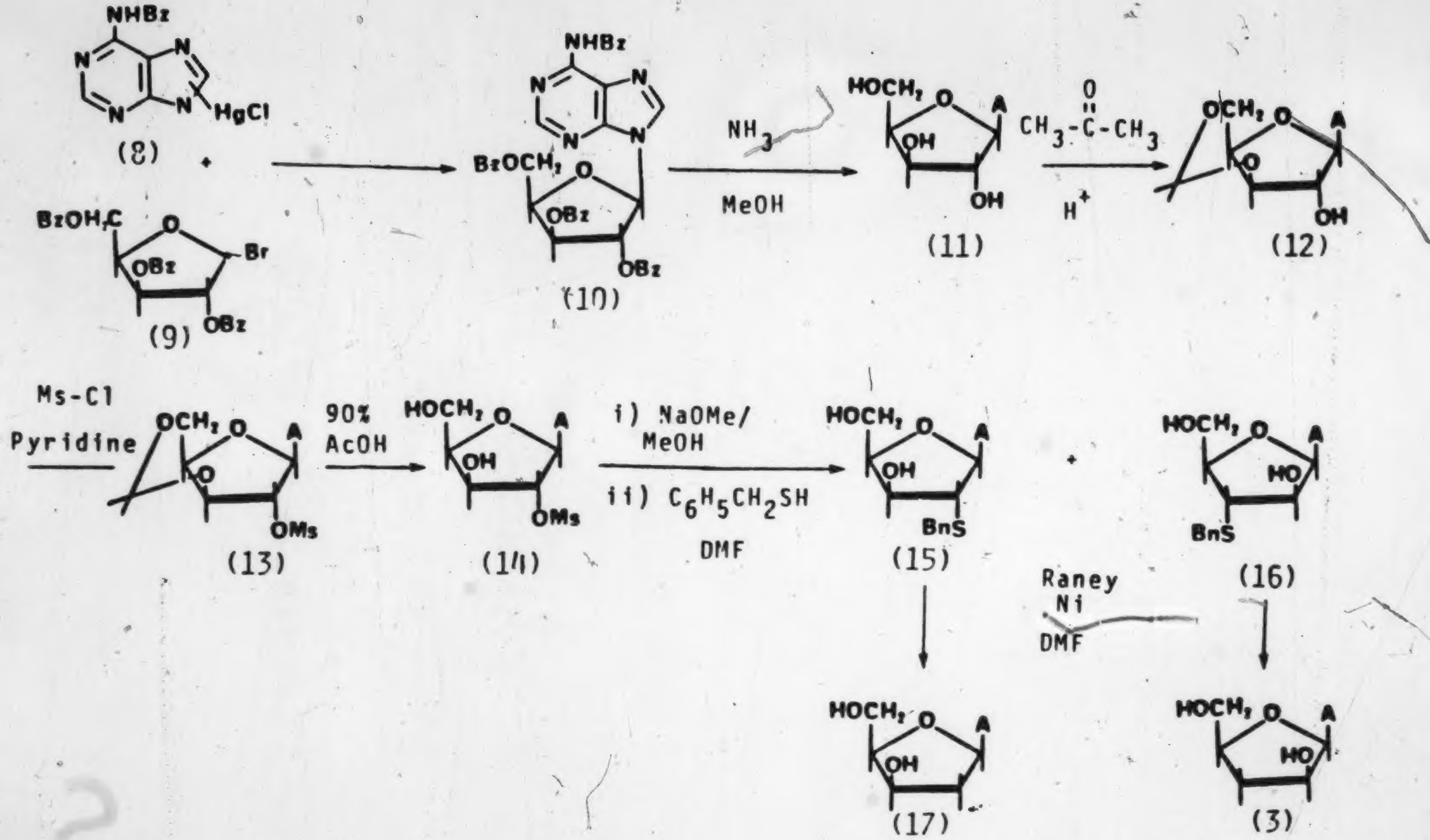
The use of fraudulent nucleosides as chemotherapeutic agents is well established,[81-83] but unfortunately these,[82,83] as with other chemotherapeutic agents,[84] do not possess a high degree of selectivity and inevitably result in drugs with relatively low margins of safety towards non-target cells. The behaviour of agrocin 84 as a cell-specific fraudulent nucleotide with separate substituents controlling toxicity and specificity is therefore an important natural guideline for future chemotherapeutic research.

RESULTS AND DISCUSSION

A. Syntheses of 9-(3-deoxy-β-D-*threo*-pentofuranosyl)-adenine (3) and 9-(2-deoxy-β-D-*threo*-pentofuranosyl)-adenine (17).

The first synthesis of these so-called 'fraudulent' nucleosides was carried out by Goodman and coworkers[79] (Scheme II). Condensation of 2,3,5-tri-*O*-benzoyl-D-xylofuranosyl bromide (9) with chloromercuri-6-benzamidopurine (8) provided 6-benzamido-9-(2,3,5-tri-*O*-benzoyl-β-D-xylofuranosyl)purine (10).[85] Removal of the benzoyl groups with methanolic ammonia gave 9-(β-D-xylofuranosyl)adenine (11). The latter compound (11) was converted to 9-(3,5-*O*-isopropylidene-β-D-xylofuranosyl)-adenine (12) which was treated with methanesulfonyl chloride to give 9-(3,5-*O*-isopropylidene-2-*O*-methanesulfonyl-β-D-xylofuranosyl)adenine (13). Deacetonation was performed with 90% aqueous acetic acid to provide 9-(2-*O*-methanesulfonyl-β-D-xylofuranosyl)-adenine (14).[86] Treatment of (14) with sodium methoxide followed by sodium benzylmercaptide gave a mixture of 2'-benzylthio (15) and 3'-benzylthio (16) nucleosides in a

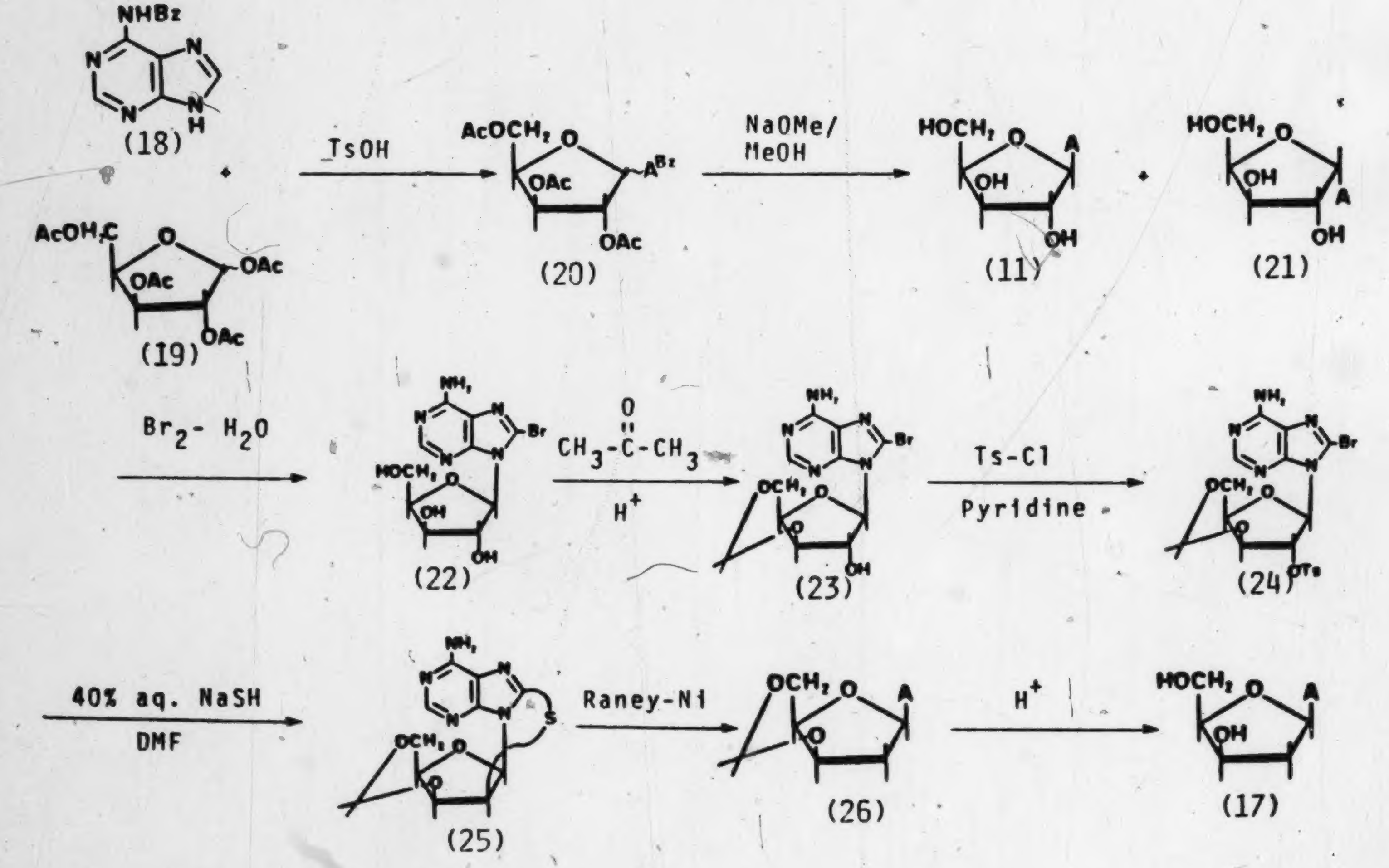
SCHEME II


NHBz
N
N
N
N
HgCl
(8)
+
BzOH$_2$C
O
Br
OBz
OBz
(9)
NHBz
BzOCH$_2$
O
OBz
OBz
(10)
NH$_3$
MeOH
HOCH$_2$
O
A
OH
OH
(11)
CH$_3$-C(=O)-CH$_3$
H$^+$
OCH$_2$
O
A
O
OH
(12)
Ms-Cl
Pyridine
OCH$_2$
O
A
O
OMs
(13)
90% AcOH
HOCH$_2$
O
A
OH
OMs
(14)
i) NaOMe/MeOH
ii) C$_6$H$_5$CH$_2$SH
DMF
HOCH$_2$
O
A
OH
BnS
(15)
+
HOCH$_2$
O
A
HO
BnS
(16)
Raney Ni
DMF
HOCH$_2$
O
A
OH
(17)
HOCH$_2$
O
A
HO
(3)

ratio of about 5:1. Separation of the two isomers followed by desulfurization with Raney nickel provided 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3) and 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (17).

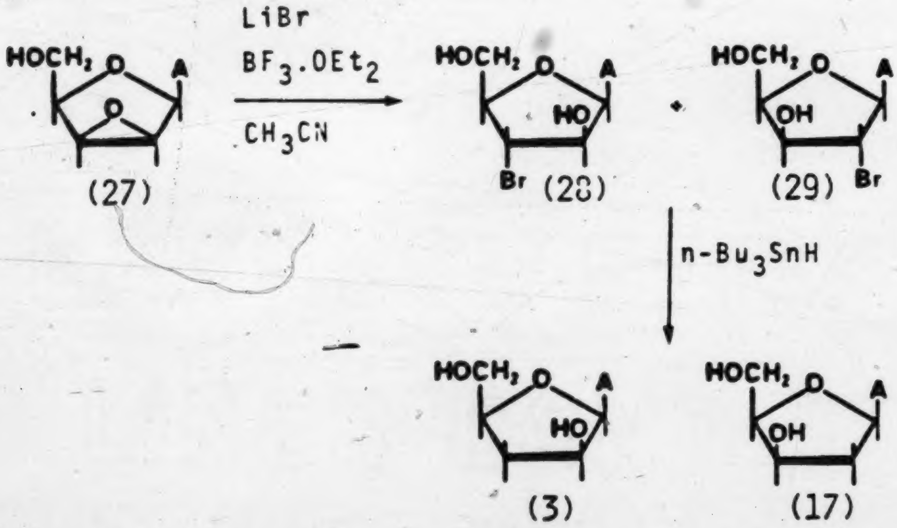
A synthesis of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (17) was reported by Ikehara[87] (Scheme III). Condensation of 1,2,3,5-tetra-*O*-acetyl-D-xylofuranose (19) with 6-*N*-benzoyladenine (18) provided an anomeric mixture of 6-benzamido-9-(2,3,5-tri-*O*-acetyl-D-xylofuranosyl)purines (20). Without separation, this mixture was treated with sodium methoxide in methanol to remove the protecting groups. The 9-β-D-xylofuranosyladenine (11) thereby obtained was halogenated at position 8 with bromine-water in an acetate buffer to give crystalline 8-bromo-9-β-D-xylofuranosyladenine (22) in 62.5% yield. In order to introduce a good leaving group at the 2'-OH, compound (22) was converted to its 3',5'-*O*-isopropylidene derivative (23) and tosylated to give 8-bromo-9-(2-*O*-tosyl-3,5-*O*-isopropylidene-β-D-xylofuranosyl)adenine (24). Treatment of (24) with a 40% aqueous solution of sodium hydrogensulfide in DMF gave the crystalline cyclonucleoside compound (25). Desulfurization of (25) with Raney nickel followed by acid catalyzed deprotection provided the desired 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (17).

SCHEME III


NHBz
(18)
+
AcOH₂C
OAc
(19)
TsOH
AcOCH₂
A^Bz
(20)
NaOMe/
MeOH
HOCH₂
A
OH
(11)
+
(21)
Br₂- H₂O
NH₂
Br
(22)
CH₃-C-CH₃
H⁺
OCH₂
(23)
Ts-Cl
Pyridine
OTs
(24)
40% aq. NaSH
DMF
S
(25)
Raney-Ni
(26)
H⁺
(17)

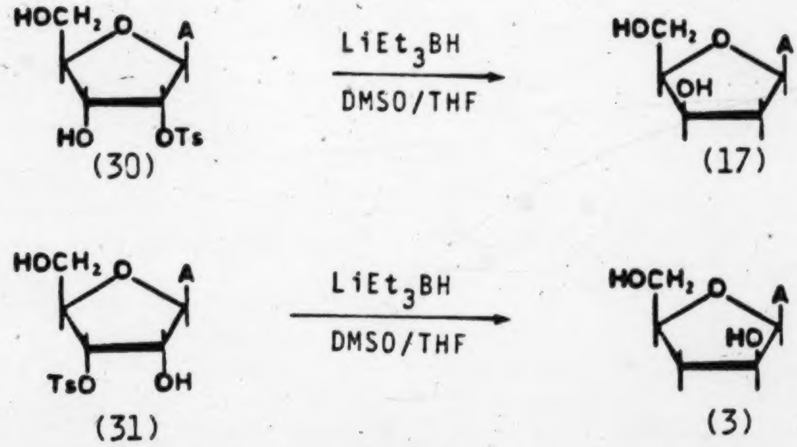
Another synthesis of the two 'fraudulent' nucleosides (3) and (17) was reported by Mengel and Wiedner[88] (Scheme IV). Oxirane ring opening of 9-(2,3-anhydro-β-D-ribofuranosyl)adenine (27)[89] with boron trifluoride etherate and lithium bromide gave a mixture of 3'- and 2'-bromo nucleosides (28) and (29). These bromohydrins were hydrogenolyzed with tributyltin hydride to give 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3) and 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (17).

SCHEME IV



Hansske and Robins[90] have reported the synthesis of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (17) via a high yield stereoselective deoxygenative rearrangement of 2'-*O*-tosyladenosine (30) (Scheme V). This [1,2]-hydride shift rearrangement proceeded smoothly at ambient temperature by using excess lithium triethylborohydride (LTBH) in THF or DMSO/THF solutions. The generality of this rearrangement was found to include the isomeric 3'-*O*-tosyladenosine (31)[91] which under identical conditions gave 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3).

SCHEME V

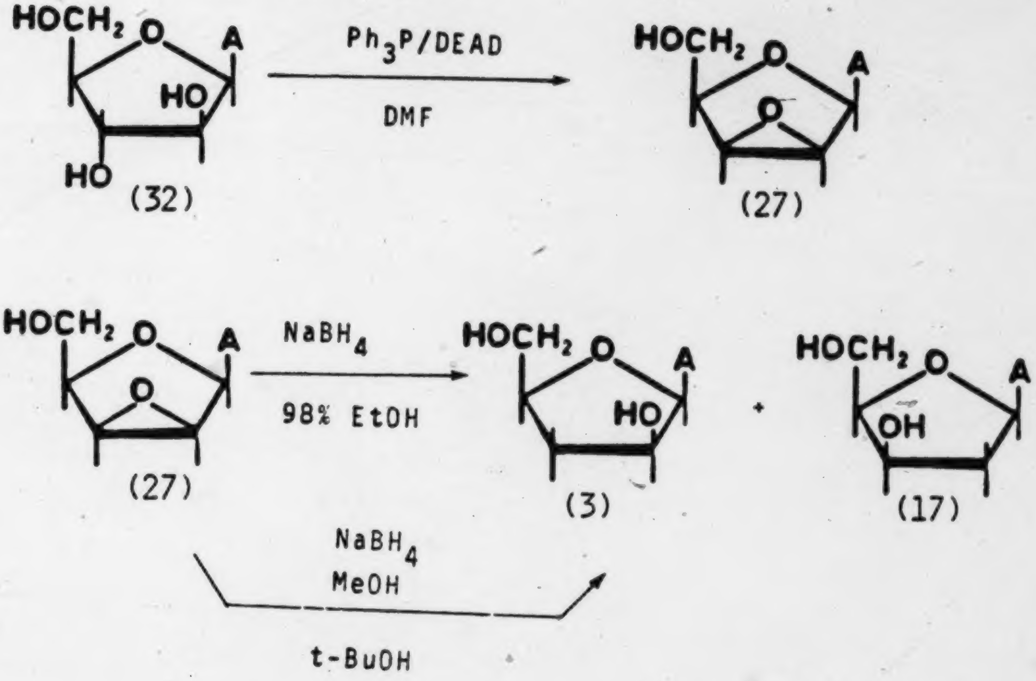


Our approach to the synthesis of the "core nucleoside" of agrocin 84 (1) is outlined in Scheme VI. Thus 9-(β-D-arabinofuranosyl)adenine (32) was converted to 9-(2,3-anhydro-β-D-lyxofuranosyl)adenine (27)[86,89,92-94] by reaction with the triphenylphosphine (TPP)-diethyl azodicarboxylate (DEAD) system.[95] This reaction was reported by Mengel and Bartke[93] to proceed to completion in dioxane at 70°C for 50 minutes and give the lyxo-epoxide (27) in 90% yield. However, in our hands only a 56% yield of (27) was obtained employing dioxane as solvent. Similar results were observed with acetonitrile as solvent. Guthrie[96] reasoned that since epoxidation takes place by nucleophillic displacement, it might be facilitated by a polar aprotic solvent such as DMF. He employed this system in synthesizing methyl 3,4-anhydro-β-D-tagatofuranoside from methyl β-D-fructofuranoside. We also found TPP/DEAD in DMF to be the system of choice and (27) could be obtained in repeatable good yields of 77-80% with no detectable side products. A convenient aspect of this TPP/DEAD reaction is that the 5'-OH group does not need protection during epoxidation. The epoxide (27) is presumably formed specifically via the 3'-oxyphosphonium intermediate (34) on the less hindered α-face, after initial reaction between TPP and DEAD to form the betaine (33)[97-99] (Scheme VII).

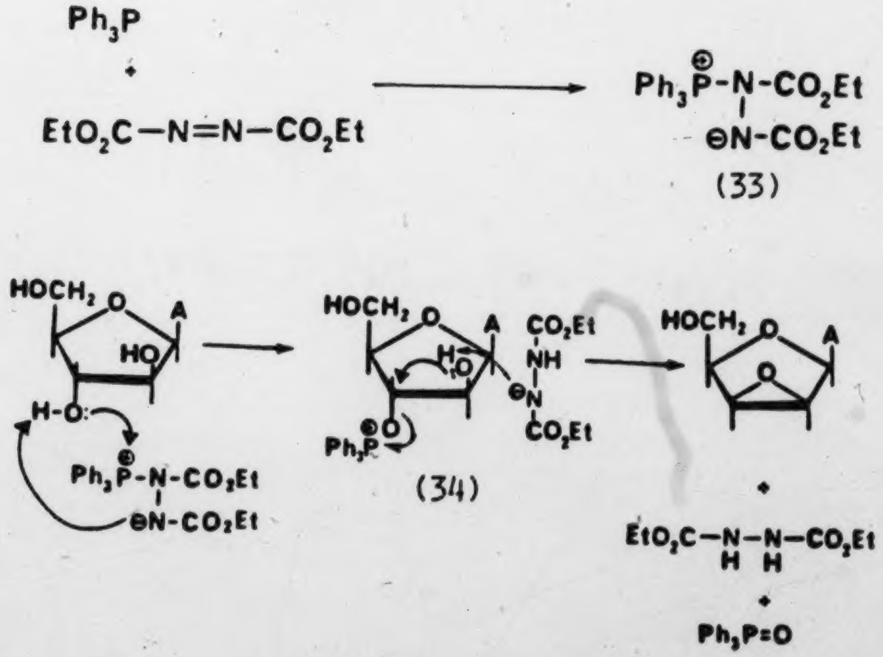
The reduction of the epoxy-nucleoside (27) was effected by refluxing it with excess sodium borohydride in 98% ethyl alcohol. The reduction proceeded in excellent yield with high regioselectivity. The predominant product (84% yield) was the desired "core nucleoside" 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3). The other regioisomer, 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (17), was obtained in 11% yield.

We also investigated the reduction of epoxy-nucleoside (27) with sodium borohydride in *t*-butyl

SCHEME VI



SCHEME VII

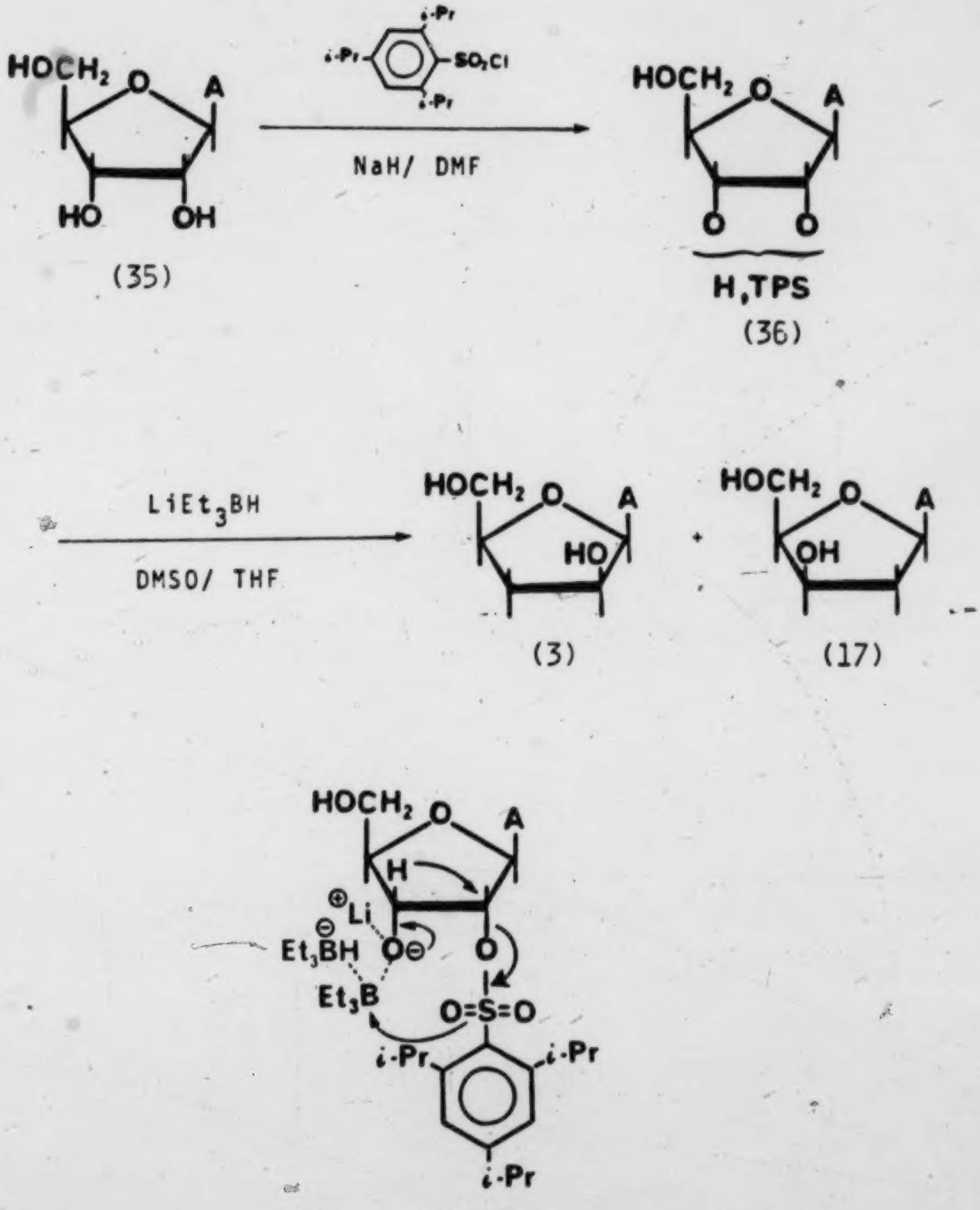


alcohol-methyl alcohol which was reported to give excellent functional selectivity.[100] This procedure also gave the desired "core nucleoside" (3) as the predominant regioisomer. However, some starting material remained unreacted, so the $NaBH_4$/98% EtOH system was utilized. The separation of the regioisomers (3) and (17) was conveniently effected by ion-exchange chromatography on Dowex 1X2 (OH^-) resin, as described by Dekker.[101]

In another approach (Scheme VIII) to the desired "core nucleoside" (3), adenosine (35) was treated with sodium hydride and 2,4,6-triisopropylbenzenesulfonyl chloride. This gave a mixture of 2'- and 3'-O-TPS-adenosines (36) in 80% yield. Separation of this regioisomeric mixture (36) was not attempted. Our [1,2]-hydride shift rearrangement[90] with (36) using LTBH in DMSO/THF solution gave 9-(3-deoxy-β-D-threo-pentofuranosyl)adenine (3) and 9-(2-deoxy-β-D-threo-pentofuranosyl)-adenine (17) in 55% yield in a ratio of 1:2. The desulfonyloxylation of 2'-O-TPS-adenosine to (17) probably occurs via a "concerted" sequence (as in 37)[90] with hydride transfer from a second borohydride species. Desulfonyloxylations of some secondary p-toluenesulfonates of glycosides by LTBH leading to 2- and 3-deoxy sugars have been reported.[102]

In another approach (Scheme IX) to the "core nucleo-

SCHEME VIII



(35)

(36)

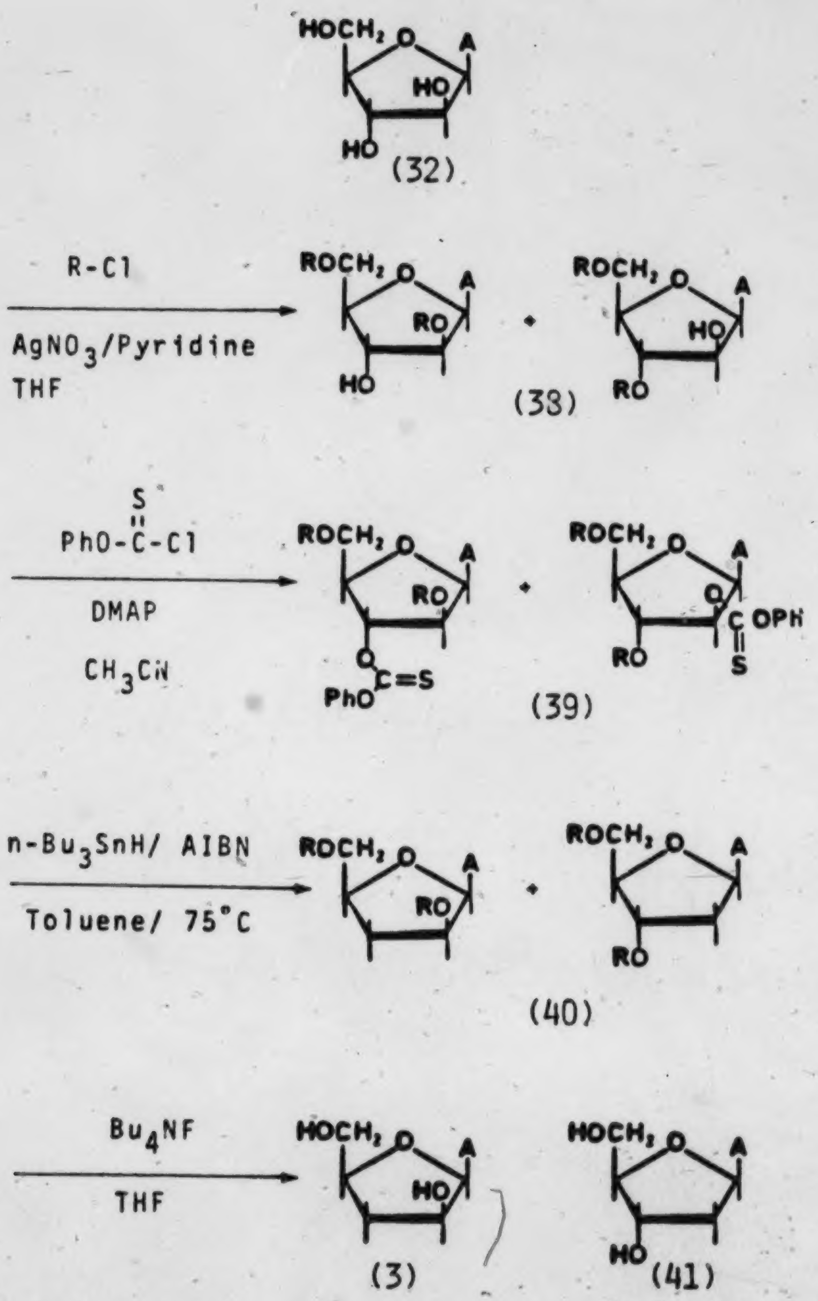
(3)

(17)

(37)

side" 9-(β-D-arabinofuranosyl)adenine (32) was subjected to silyation with t-butyldimethylsilyl chloride/$AgNO_3$/ pyridine to give a mixture of 2',5'-di-O- and 3',5'-di-O-silylated arabinosyladenines (38) in a ratio of 10:3 (as determined by NMR). This is in contrast to the 92:3 regioselectivity reported by Ogilvie et al.[103] Separation of this mixture was not attempted since the two regioisomers had extremely close R_f values (silica gel, 5% MeOH/$CHCl_3$). It was subjected to a deoxygenation procedure developed earlier in our laboratories.[104,105] Thus reaction of (39) with phenyl chlorothionocarbonate in the presence of 4-dimethylaminopyridine (DMAP) in acetonitrile proceeded in good yield to give the 2',5'-di-O-(t-butyldimethylsilyl)-3'-O-phenoxythiocarbonyl- and 3',5'-di-O-(t-butyldimethylsilyl)-2'-O-phenoxythiocarbonylarabinosyladenines (39). Excess DMAP was required for this reaction as it was found in initial experiments that some (38) remained unreacted (presumably due to the hindered arabino 2'-OH). Reductive cleavage of (39) occurred readily with tri-n-butylstannane in toluene at 75°C with α,α'-azobisisobutyronitrile as initiator. Deprotection of the silyl ethers was effected by tetra-n-butylammonium fluoride at room temperature. Chromatographic separation on Dowex 1X2 (OH^-) resin gave 2'-deoxyadenosine (41) and the desired 9-(3-deoxy-β-D-

SCHEME IX



(32)

(38)

(39)

(40)

(3) (41)

R= TBDMS-

threo-pentofuranosyl)adenine (3) in a ratio of 1:5 and an overall yield of 37% from arabinosyladenine (32).

Of the various methods described for the synthesis of 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3), the "core nucleoside" of agrocin 84 (1), the one described in Scheme VI is best when one considers the combined features of selectivity, safety, yield, and time. Methods described by Goodman,[79] Ikehara,[87] Mengel,[88] and the one in Scheme IX are multistage processes leading eventually to low overall yields. Although the rearrangement of 3'-*O*-tosyladenosine (31) proceeds in 82% yield[90] it requires preparation of the rather difficultly accessible 3'-*O*-tosyladenosine starting material and involves the use of lithium triethylborohydride. Caution is required during work-up of this reaction (and also that in Scheme VIII). In contrast, Scheme VI employes cheap common reagents and easy work-up procedures. Its only drawback is the use of 9-(β-D-arabinofuranosyl)adenine (32) as starting material.

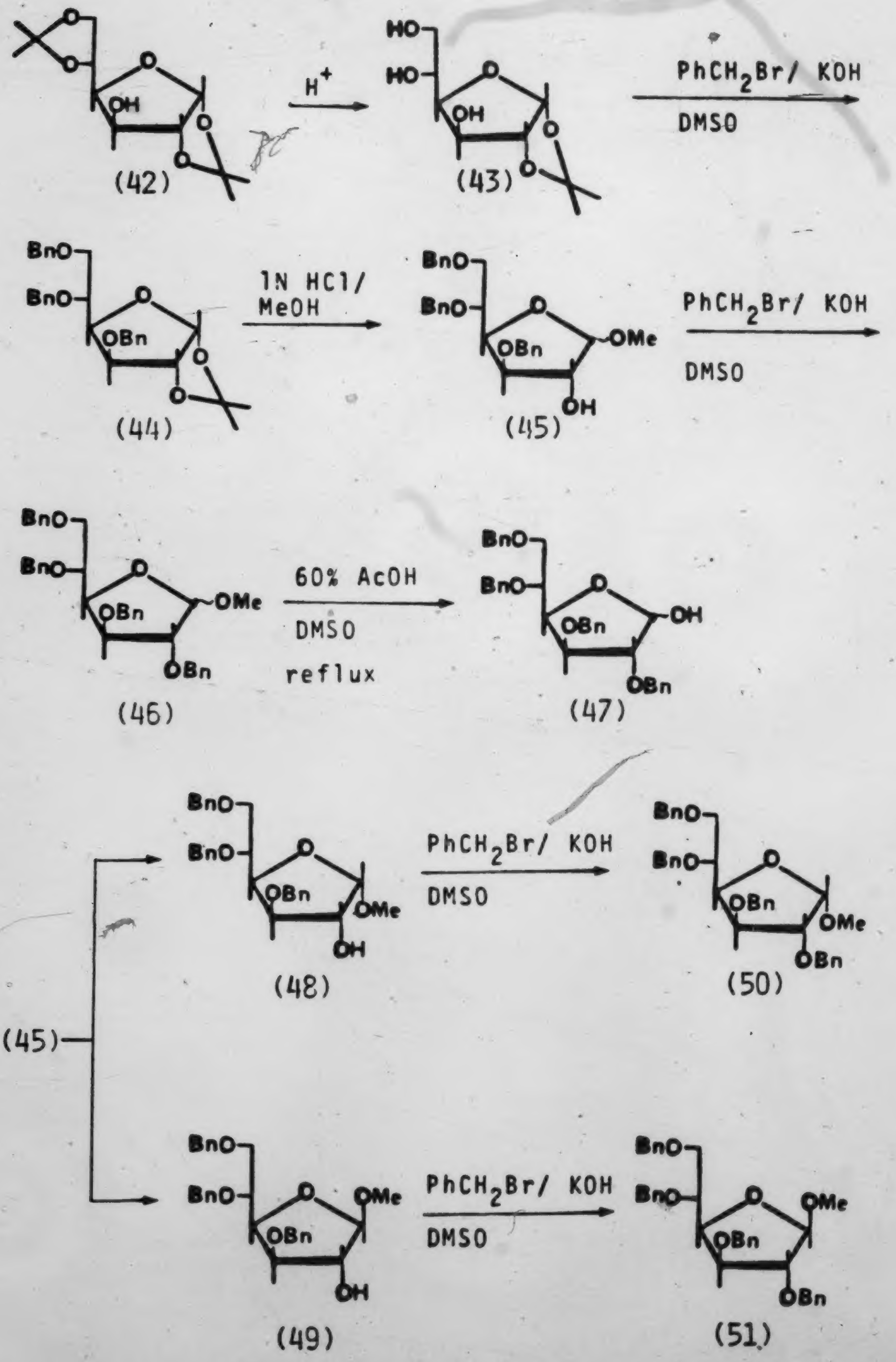
B. Synthesis of 2,3,5,6-tetra-O-benzyl-D-glucofuranose (47)

The structural evidence for agrocin 84 (1) suggests the presence of a D-glucofuranosyloxyphosphoryl substituent at N6 of adenine.[63-65] Hence, we decided to synthesize the title compound as the most favorable precursor for this phosphoramidate linkage.

Potassium hydroxide in combination with benzyl chloride is used frequently for the benzylation of carbohydrates[106] with [107-109] or without[110,111] solvents such as dioxane[107,108] or toluene.[109] However, this method of benzylation has proved to be impractical in some instances.[111,112] The use of benzyl bromide and potassium hydroxide in DMSO considerably enhances the rate of benzylation, yields are significantly improved, and benzylation of unreactive and hindered hydroxyl groups is made possible.[113] This procedure is advantageous relative to other methods used in carbohydrate chemistry since it is fast, takes place under mild conditions, uses economical and stable commercially available reagents, and gives resulting products in high yields. An additional advantage is that no special precautions are necessary against moisture.[114] Other O-benzylation methods used in carbohydrate chemistry require carefully dried solvents[115,116] and/or light sensitive reagents,[117] long reaction times etc.

1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (42) was converted quantitatively to 1,2-O-isopropylidene-α-D-glucofuranose (43) by the published procedure[118] (Scheme X). This compound (43) was converted to 3,5,6-tri-O-benzyl-1,2-O-isopropylidene-α-D-glucofuranose (44)[108] by benzyl bromide and potassium hydroxide in DMSO. Huber and Rossi[108] have reported the preparation of (44) from (43) using benzyl bromide and potassium hydroxide in dioxane. Change of solvent from dioxane to DMSO greatly enhanced the rate of benzylation and the reaction proceeded smoothly at room temperature. Compound (44) was converted quantitatively to methyl 3,5,6-tri-O-benzyl-α,β-D-glucofuranose (45)[108] by hydrogen chloride in methanol. The essentially equimolar mixture (45), of α (48) and β (49) anomers was separated easily by column chromatography on silica gel. These two anomers were subjected separately to the above noted benzylation conditions to give methyl 2,3,5,6-tetra-O-benzyl-α-D-glucofuranoside (50) and methyl 2,3,5,6-tetra-O-benzyl-β-D-glucofuranoside (51), respectively, in virtually quantitative yields. Compounds (50) and (51) were distinguished by ^{1}H NMR. The peak for H-1 of (51) appeared at δ 4.9 and that of (50) at

SCHEME X


H+
(42)
(43)
PhCH2Br/ KOH
DMSO
1N HCl/
MeOH
(44)
(45)
PhCH2Br/ KOH
DMSO
60% AcOH
DMSO
reflux
(46)
(47)
(45)
PhCH2Br/ KOH
DMSO
(48)
(50)
PhCH2Br/ KOH
DMSO
(49)
(51)
HO
OH
BnO
OBn
OMe

δ 5.02. In the former (51), H-1 is shielded by the cis vicinal benzyl group. J_{1-2} for the α anomer is 4 Hz whereas there is no J_{1-2} coupling observed for the β anomer. Compounds (50) and (51) have identical R_f values (0.67) on silica gel TLC in $CHCl_3$/acetone (95/5), whereas compound (48) has R_f 0.5 and (49) has R_f 0.3 with the same solvent system.

Methyl 3,5,6-tri-O-benzyl-α,β-D-glucofuranoside (45) was benzylated to give methyl 2,3,5,6-tetra-O-benzyl-α,β-D-glucofuranoside (46). Glycosyl hydrolysis was effected with 60% aqueous acetic acid in DMSO to yield 2,3,5,6-tetra-O-benzyl-D-glucofuranose (47) with an α:β ratio of 2:3. Rossi[108] had used 60% aqueous acetic acid to convert ethyl 2-O-methyl-3,5,6-tri-O-benzyl-β-D-glucofuranoside to 2-O-methyl-3,5,6-tri-O-benzyl-α,β-D-glucofuranose. However, we found that (46) is insoluble in 60% aqueous acetic acid and had to use DMSO as the co-solvent to obtain a homogeneous solution.

The α and β anomers of 2,3,5,6-tetra-O-benzyl-D-glucofuranose (47) were distinguished by ^1H NMR. The peak for H-1 of the α-anomer appears at δ 5.35 whereas the chemicals shift for H-1 of the β-anomer is δ 5.13. Also, the chemical shift for the hydroxyl proton of the α-anomer is δ 6.25 whereas that for the β-anomer is δ 6.50 (downfield relative to the α-anomer whose hydroxyl proton is shielded by the cis vicinal benzyl group).

C. Synthesis of DL-threo-2,3-dihydroxy-4-methylpentanamide (7')

Structural evidence for agrocin 84 (1) indicates the presence of a D-threo-2,3-dihydroxy-4-methylpentanamide (7) unit which is linked to the 5'-hydroxyl group of the nucleoside through a phosphoramidate linkage.[63-65] This compound (7) has been obtained[64] from racemic threo-2,3-dihydroxy-4-methylpentanoic acid.[119] This in turn was obtained in very poor yield from the α,β-unsaturated isohexenoic acid by permanganate oxidation. The racemic acid was resolved as its quinine salt and the dextrorotatory acid ($[\alpha]_D = +13.6 \pm 0.2$) was converted via its methyl ester to the required levorotatory amide ($[\alpha]_D = -52.9 \pm 0.6$).

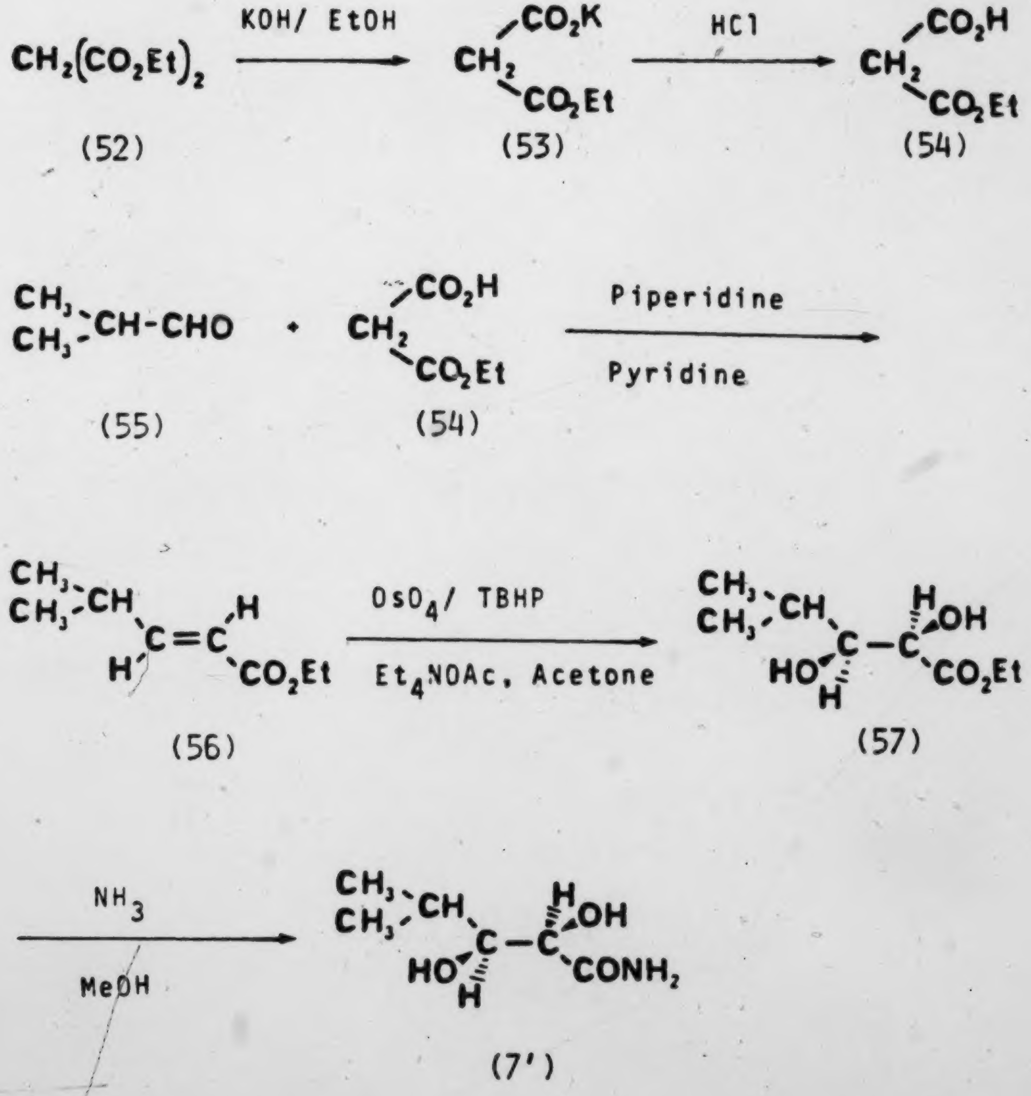
Our approach to the synthesis of the title compound (7') (Scheme XI) began with conversion of diethyl malonate (52) into the monopotassium salt (53)[120] which was converted to the free acid (54)[120] by hydrochloric acid. Knoevenagel condensation[121-123] of (54) with isobutyraldehyde (55) gave ethyl (E)-4-methylpent-2-enoate (56)[124-126] in 67% yield. This condensation reaction was run in pyridine solution (the Doebner modification) with piperidine as the catalyst.

Decarboxylation occurred in the reaction mixture as expected to give the α,β-unsaturated ester (56) directly.

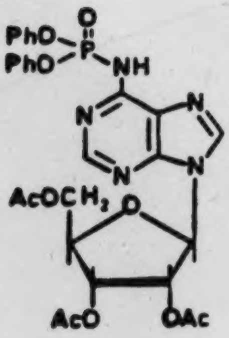
The t-butyl hydroperoxide (TBHP)-based osmium-catalyzed procedure for vicinal dihydroxylation of olefins[127,128] was used to convert the (E)-ester (56) to ethyl DL-threo-2,3-dihydroxy-4-methylpentanoate (57). This is more reliable than earlier chlorate (Hoffmann[129]) and hydrogen peroxide (Milas[130]) based osmium catalyzed procedures. According to Sharpless the key factor in the new method appears to be the presence of a nucleophile (either $Et_4N^+\bar{O}H$[127] or $Et_4N^+\bar{O}Ac$[128]), which likely increases the turnover rate of the catalytic cycle by facilitating removal of the glycol product from the coordination sphere of osmium.

The dihydroxy ester (57) was converted to DL-threo-2,3-dihydroxy-4-methylpentanamide (7') by methanolic ammonia in 72% yield.

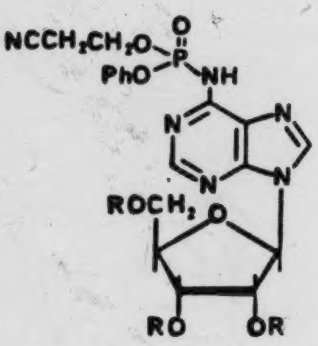
SCHEME XI


KOH/ EtOH
HCl
(52)
(53)
(54)
Piperidine
Pyridine
(55)
(54)
OsO4/ TBHP
Et4NOAc, Acetone
(56)
(57)
NH3
MeOH
(7')

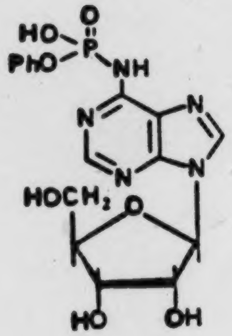
D. Nomenclature for 6-*N*-(phosphoryl)adenosines



(58)



(59) R= Ac
(60) R= H



(61)

The synthesis of compound (58) has been reported by Charubala and Pfleiderer.[131] This compound was named by them as 2',3',5'-tri-*O*-acetyl-N^6-diphenylphosphoryladenosine.

The same compound has been named by Chemical Abstracts as phosphoramidic acid, *N*-[9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9H-purin-6-yl]-diphenyl ester.

Similarly Charubala and Pfleiderer have designated compound (59) as 2',3',5'-tri-*O*-acetyl-N^6-cyanoethylphenyl-phosphoryladenosine and its deacetylated derivative (60) as N^6-cyanoethyl-phenylphosphoryl-adenosine.

Compounds (59) and (60) have been named by Chemical Abstracts as phosphoramidic acid, N-[9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-9H-purin-6-yl]-2-cyanoethyl phenyl ester and Adenosine, N-[(2-cyanoethoxy)phenoxyphosphinyl]-.

It can be seen that compounds (59) and (60) are structurally identical, with (59) being the triacetyl derivative of (60). However, Chemical Abstracts has named the former as a phosphoramidic acid and the latter as a substituted adenosine. Extensions of these types of designations by Chemical Abstracts to structures containing a richer variety of substituents will lead to greater confusion.

We therefore suggest two types of nomenclature for these types of compounds. Compounds 58-61 will be used as representative examples.

"Preferred" systematic Nomenclature

(58) Diphenyl N-[9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-9H-purin-6-yl]phosphoramidate.

(59) 2-Cyanoethyl phenyl N-[9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-9H-purin-6-yl]phosphoramidate.

(60) 2-Cyanoethyl phenyl N-[9-(β-D-ribofuranosyl)-9H-purin-6-yl]phosphoramidate.

(61) Phenyl N-[9-(β-D-ribofuranosyl)-9H-purin-6-yl]phosphoramidic acid.

Systematic Derivative Nomenclature

(58) 2',3',5'-Tri-O-acetyl-6-N-(diphenoxyphosphinyl)-adenosine.

(59) 2',3',5'-Tri-O-acetyl-6-N-[(2-cyanoethoxy)phenoxyphosphinyl]adenosine.

(60) 6-N-[(2-Cyanoethoxy)phenoxyphosphinyl]adenosine.

(61) 6-N-(Hydroxyphenoxyphosphinyl)adenosine.

We also suggest the following informal nomenclature for these type of compounds which will be used in this dissertation.

(58) 2',3',5'-Tri-O-acetyl-6-N-(diphenylphosphoryl)-adenosine.

(59) 2',3',5'-Tri-O-acetyl-6-N-[(2-cyanoethyl)phenylphosphoryl]adenosine.

(60) 6-N-[(2-Cyanoethyl)phenylphosphoryl]adenosine.

(61) 6-N-(Phenylphosphoryl)adenosine.

E. Syntheses of 6-N-(phosphoryl)adenosine derivatives

Since phosphomono- and diesters are ionized acidic species at physiological pH they do not normally penetrate cell walls[132] and therefore have little or no effect on intracellular biological systems. Moreover, such compounds are subject to degradation by extracellular hydrolytic enzymes that convert them to nucleosides. The transport barrier of the cell wall might be overcome by the use of certain electroneutral phosphotriesters. They also might be stable to various hydrolytic enzymes.

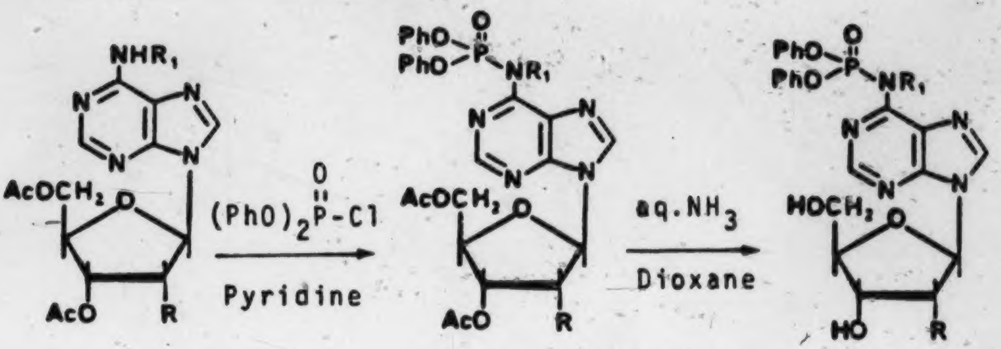
Pfleiderer[131] has synthesized several trisubstituted phosphoesters of adenosine and 2'-deoxyadenosine with the phosphoester function located at positions 3',5' and N6. Diphenyl phosphorochloridate reacted smoothly with 3',5'-di-O-acetyl-2'-deoxyadenosine (62) to give compound (63) which was deacetylated by aqueous ammonia in dioxane to give (64). Analogous reactions with 2',3',5'-tri-O-acetyladenosine (65) also proceeded smoothly. To determine the location of phosphorylation a similar reaction was carried out with 2',3',5'-tri-O-acetyl-6-N-(methyl)adenosine (67).

The N6 phosphorylation reaction was extended to other phosphorylating agents. Bis-2-chlorophenyl phosphorochloridate reacted with (65) and (67) to give the diesters (70) and (72). Deacetylation afforded the 6-N-(phosphoryl)adenosine derivatives (71) and (73). The synthesis of N6 phosphoramidate diesters with two different ester functions was also accomplished by Charubala and Pfleiderer[131] by reaction of 2',3',5'-tri-O-acetyladenosine (65) with phenyl- and 2-chlorophenyl phosphorodichloridate, respectively, and subsequent treatment of the resulting intermediate with 2-cyanoethanol. For the synthesis of compound (74) the initial reaction was carried out in the presence of 1,2,4-triazole. Treatment of compounds (59), (70), (72) and (74) with aqueous ammonia in dioxane removed the acetyl groups but left the phosphoramidate diester intact.

Charubala and Pfleiderer[131] noted that the acidity of the phosphoramidate group (pka ~7) could oppose the normally facile base-promoted β-elimination of the 2-cyanoethyl group. If the deacetylation was extended for longer times, traces of adenosine and other side products were detected.

Agrocin 84 (1) contains a (D-glucofuranosyloxy)-phosphoryl substituent which is attached to the N6 position of the modified "core nucleoside" (3).[63-65] Model studies on joining the phosphorus and nucleoside

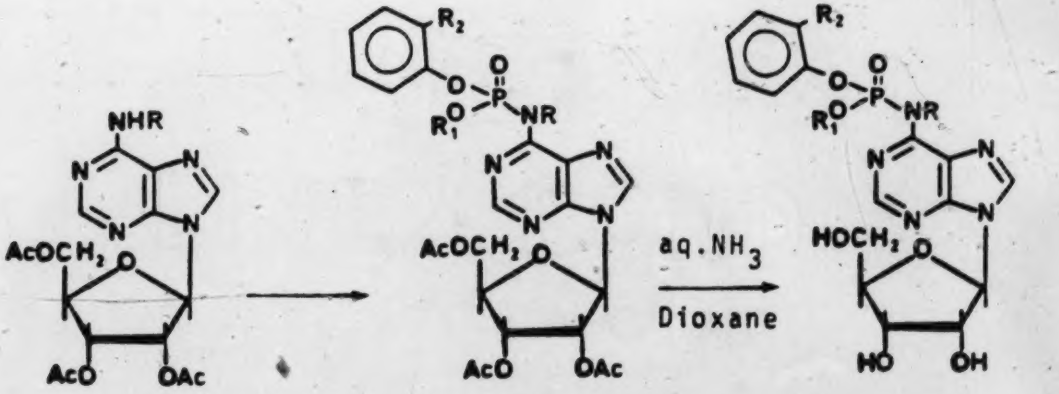
SCHEME XII



	R	R_1
62)	H	H
65)	OAc	H
67)	OAc	CH_3

	R	R_1
63)	H	H
58)	OAc	H
68)	OAc	CH_3

	R	R_1
64)	H	H
66)	OH	H
69)	OH	CH_3



	R
65)	H
67)	CH_3

	R	R_1	R_2
70)	H	2-ClPh	Cl
72)	CH_3	2-ClPh	Cl
59)	H	CH_2CH_2CN	H
74)	H	CH_2CH_2CN	Cl

	R	R_1	R_2
71)	H	2-ClPh	Cl
73)	CH_3	2-ClPh	Cl
60)	H	CH_2CH_2CN	H
75)	H	CH_2CH_2CN	Cl

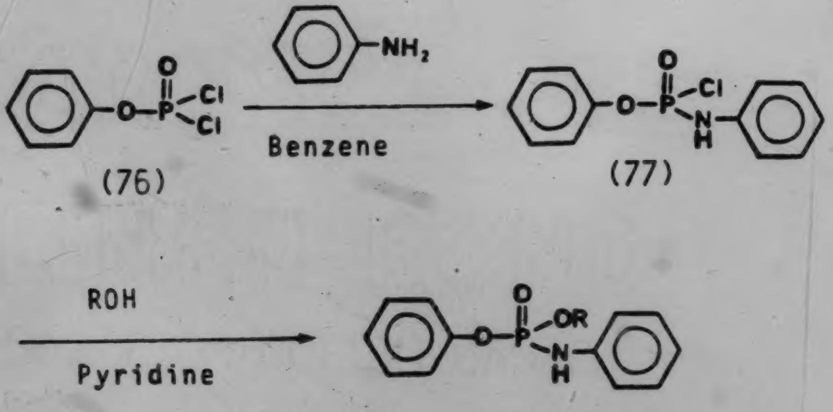
2-ClPh : 2-chlorophenyl

units to give 6-*N*-phosphoramidate compounds were undertaken. We have explored two basic approaches which lead to these types of compounds:

1) Phenyl phosphorodichloridate approach and
2) Phosphite-azide coupling approach.

1) Phenyl phosphorodichloridate approach

Zielinski and Lesnikowski[133] have reported that phenyl phosphorodichloridate (76) reacts with aniline to give phenyl *N*-phenylphosphoramidochloridate (77). This compound reacted rapidly with alcohols in pyridine at 0°C to give the *O*-alkyl-*O*-phenyl *N*-phenylphosphoramidates (78).



(76) (77) (78)

We chose 2',3',5'-tri-*O*-acetyladenosine (65)[134-136] as the model nucleoside for phosphorylation at the N6 position with phenyl phosphorodichloridate. Treatment of (65) with phenyl phosphorodichloridate in refluxing benzene afforded intermediate (79) (not isolated), which was treated with phenol to yield 2',3',5'-tri-O-acetyl-6-N-(diphenylphosphoryl)adenosine (58) in 64% yield (Scheme XIII). Compound (58) has been prepared by Charubala and Pfleiderer[131] by reaction of 2',3',5'-tri-O-acetyl-adenosine (65) with diphenyl phosphorochloridate in dry pyridine.

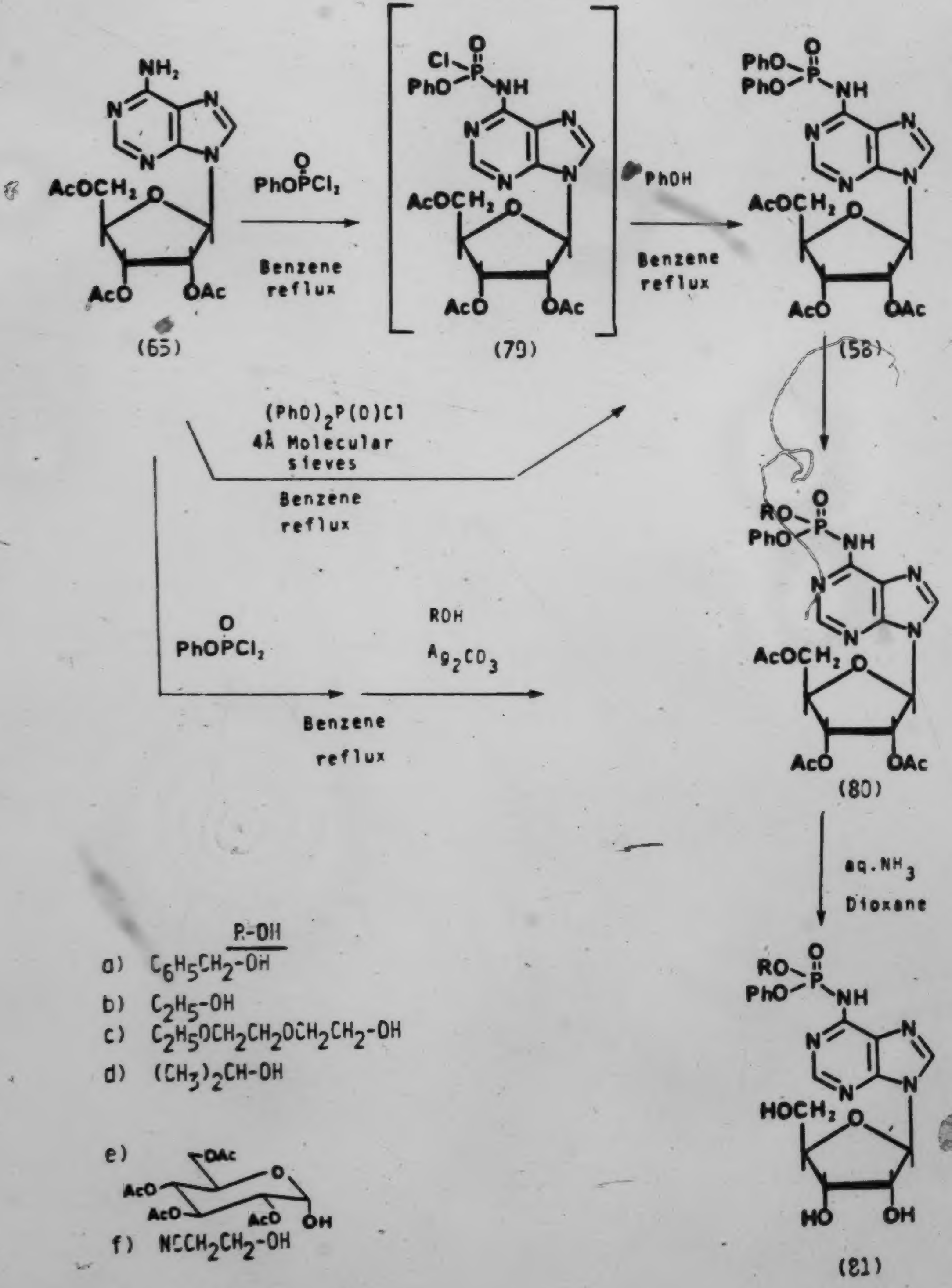
Compound (58) was obtained in 60% yield by treatment of (65) with 1.43 equivalents of diphenyl phosphorochloridate in refluxing benzene for 24 hours. The yield of this reaction was increased to 94% by inclusion of 4Å molecular sieves. The latter reaction, however, required 48 hours of reflux with 8.5 equivalents of the phosphorylating agent. In the former case, further reaction was not observed with excess diphenyl phosphorochloridate and increased reflux time. This suggests that formation of an acid salt or complex with the adenine base blocked further phosphorylation at N6. The 4Å molecular sieves are known to be effective acceptors of hydrogen chloride and bromide.[137]

Treatment of 2',3',5'-tri-O-acetyladenosine (65) with phenyl phosphorodichloridate to give intermediate (79) followed by addition of benzyl alcohol gave less than 40% yields of 2',3',5'-tri-O-acetyl-6-N-[(benzyl)phenylphosphoryl]adenosine (80a). We found that addition of silver carbonate to the reaction mixture before addition of the alcohol enhanced the yield to 63%. The generality of this enhancement was extended with other alcohols and the 2',3',5'-tri-O-acetyl-6-N-(phosphoryl)adenosine nucleosides were obtained in yields ranging from 58% (59) to 76% (80c). Compound (59) was identical in its UV spectral behaviour and other reported characteristics to 2',3',5'-tri-O-acetyl-6-N-[(2-cyanoethyl)phenylphosphoryl]adenosine.[131]

Another observation made during this series of reactions was that Merck 7734 (100-200 mesh) silica gel as received was unsuitable for chromatographic purification of these compounds. The yields of compounds (58) and (80a) were considerably lower when chromatography was carried out on this adsorbant. This was found to result from acid lability of the N-P bond of the phosphoramidate group. Neutralization of this silica gel was effected by treatment with anhydrous 1,2-dimethoxyethane saturated with ammonia at 0°C.

All of the 2',3',5'-tri-O-acetyl-6-N-(phosphoryl)adenosine nucleosides were deacetylated using aqueous ammonia in dioxane[131] to give the corresponding 6-N-(phosphoryl)adenosine nucleosides (81a-d) (Scheme XIII) in essentially quantitative yields. All the compounds were recrystallized from chloroform by diffusion of diethyl

SCHEME XIII



R-OH

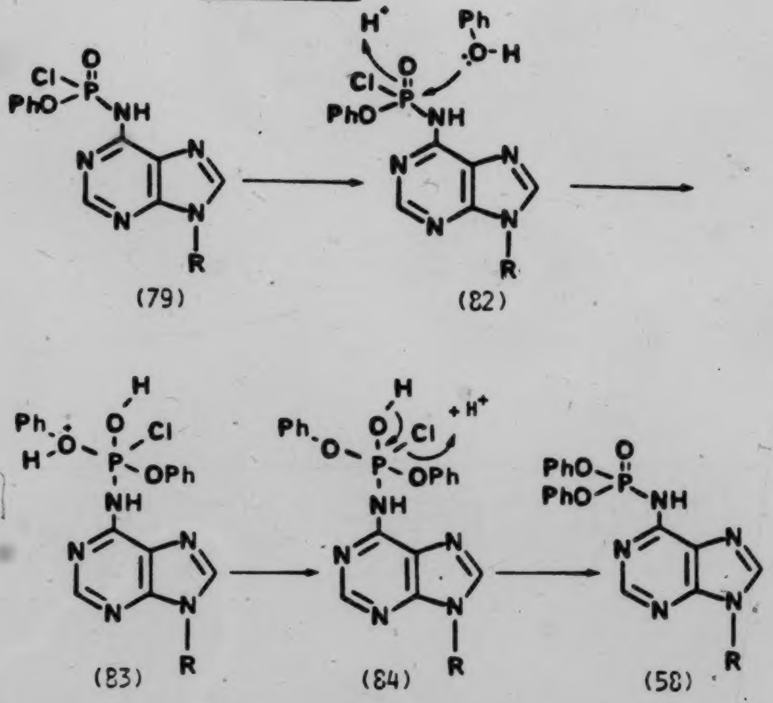
a) $C_6H_5CH_2-OH$
b) C_2H_5-OH
c) $C_2H_5OCH_2CH_2OCH_2CH_2-OH$
d) $(CH_3)_2CH-OH$
e)
f) $NCCH_2CH_2-OH$

ether. The tri-O-acetyl- and deprotected 6-N-(phosphoryl)adenosines were characterized by ^{1}H NMR and UV spectroscopy, fast atom bombardment mass spectrometry (FABMS), and elemental analyses.

The yield enhancements with addition of silver carbonate for obtaining compounds (80a-d) might be rationalized as shown in Scheme XV. Phenol (pka = 10.0) is considerably more acidic than the alcohols and reacts with (79) without addition of a base. Reaction of the alcohols with (79) may proceed via a push-pull process as depicted in Scheme XV.

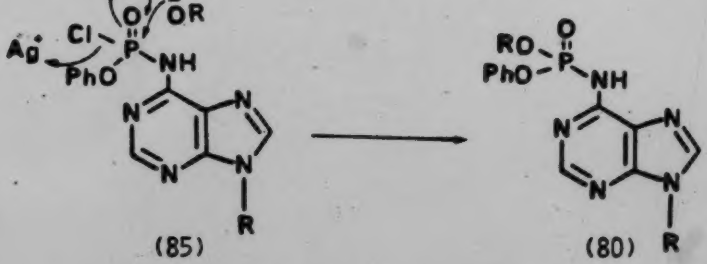
Hydrogenation of compounds (80a) and (81a) with 10% palladium/charcoal in 95% ethyl alcohol gave 2',3',5'-tri-O-acetyl-6-N-(phenylphosphoryl)adenosine and 6-N-(phenylphosphoryl)adenosine (61). The UV spectral behaviour of these latter compounds is very similar to that of agrocin 84. A discussion of the UV spectral behaviour of all the 6-N-(phosphoryl)nucleosides is presented in the following section (F) of this dissertation.

SCHEME XIV



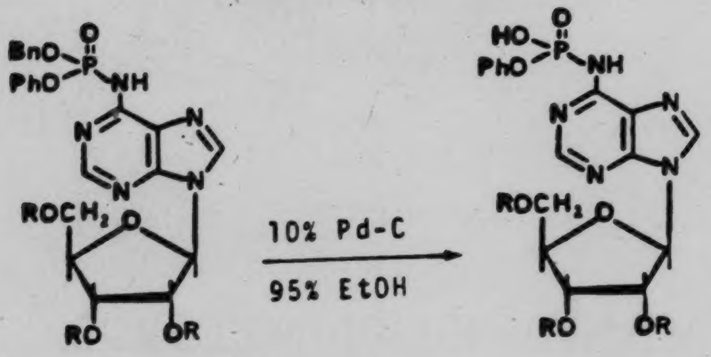
SCHEME XV

$$ROH + Ag_2CO_3 \rightleftharpoons RO^- Ag^+ + AgHCO_3$$



R= 2',3',5'-Tri-*O*-acetyl-β-*D*-ribofuranosyl-

SCHEME XVI



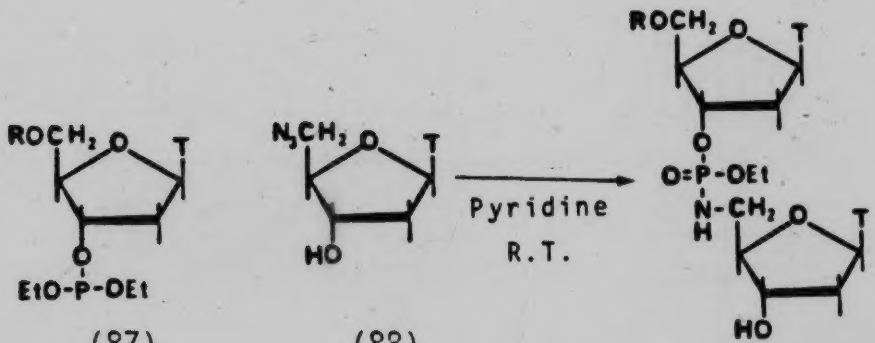
(80 a) R = Ac
(81 a) R = H

(86) R = Ac
(61) R = H

A coupling of 2,3,4,6-tetra-O-acetyl-D-glucopyranose[138] (compound e, Scheme XIII) to give compound (80e) appeared to proceed once in about 7% yield as indicated by UV spectra and FABMS, but the quantity obtained was insufficient for other analyses. Several attempts to repeat this coupling under similar conditions failed. Use of 2,3,5,6-tetra-O-benzyl-D-glucofuranose (47) as the alcohol moiety under similar conditions failed to give the expected product. Instead, the sugar decomposed to give black tarry material.

2. Phosphite-Azide Coupling Approach

5'-Azido-5'-deoxynucleosides have been reported to react with trialkyl and triaryl phosphites[139,140] to give the diesters of 5'-amino-5'-deoxynucleoside phosphoramidates. Oligoazanucleotides have been obtained in moderate yields by condensing 5'-azido-5'-deoxythymidine (88) with thymidine diethyl-3'-phosphite (87).[141] Elimination of both nucleoside and ethyl groups from the intermediate phosphoimine was observed.



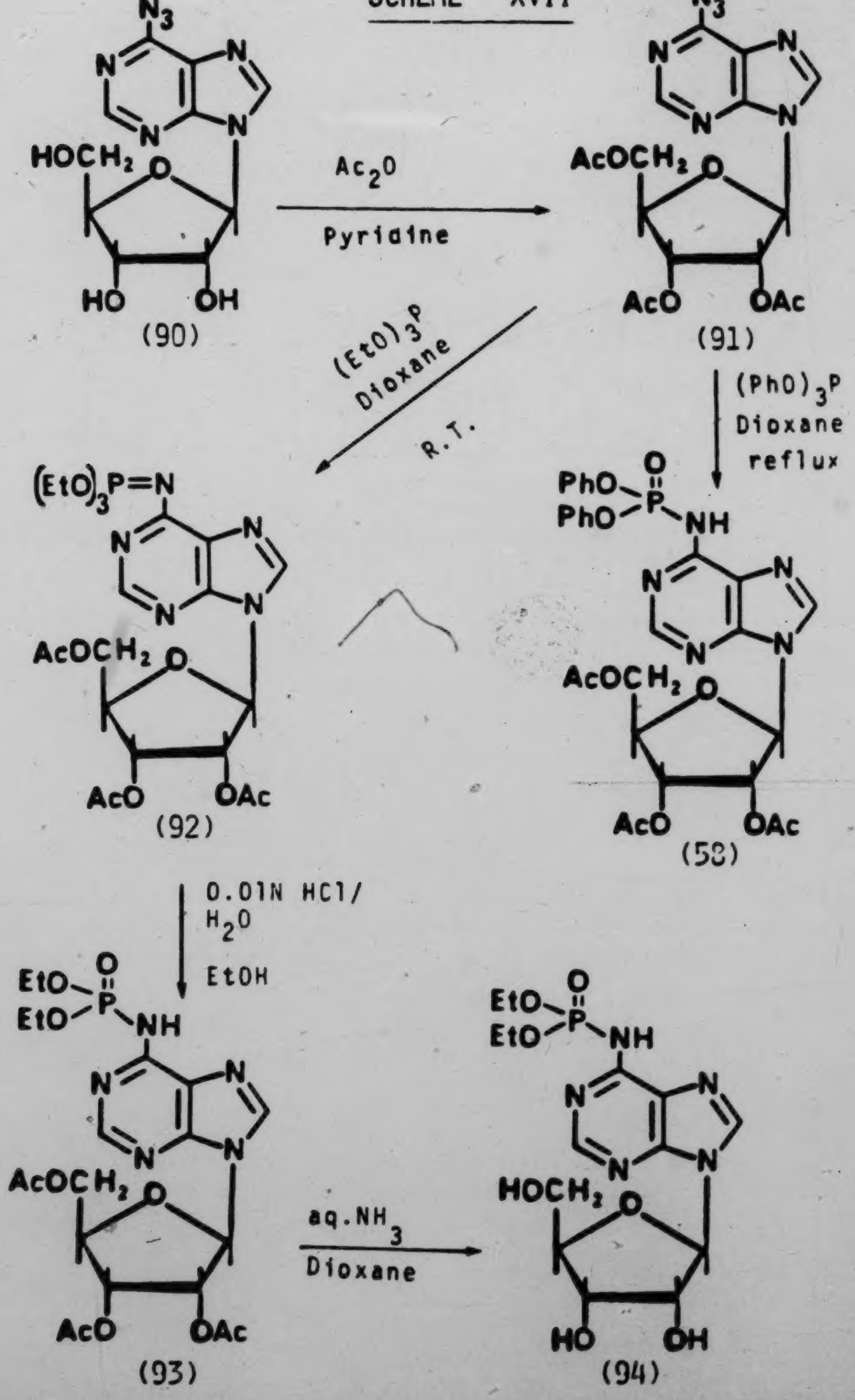
(87) (88) (89)

We decided to apply this phosphite-azide coupling approach to the synthesis of 6-*N*-phosphoryl nucleosides. 6-Azido-9-β-D-ribofuranosylpurine (90)[142,143] was converted to its tri-*O*-acetyl derivative (91) in virtually quantitative yield.

We investigated reactions of this 2',3',5'-tri-*O*-acetyl-6-azido-9-β-D-ribufuranosylpurine (91) with triethyl and triphenyl phosphite (Scheme XVII). It was observed that triethyl phosphite reacted smoothly with (91) to form the 6-*N*-phosphorimidite nucleoside (92). This reaction was monitored by UV spectroscopy since compounds (91) and (92) have extremely close TLC R_f values in the solvent systems examined. The UV spectrum of 6-azido nucleoside (91) has a λ_{max}^{MeOH} of 286 nm. Within a few minutes after the addition of triethyl phosphite the λ_{max}^{MeOH} of the reaction mixture had shifted to 269 nm and remained unchanged over the next 2 hours. When compound (92) was subjected to acidic conditions, 2',3',5'-tri-O-acetyl-6-N-(diethylphosphoryl)adenosine (93) and 2',3',5'-tri-O-acetyladenosine (65) were obtained in nearly equal amounts. Compound (93) was deacetylated by aqueous ammonia in dioxane to give 6-N-(diethylphosphoryl)-adenosine (94).

The reaction of (91) with triphenyl phosphite did not proceed as smoothly as with triethyl phosphite. As indicated by UV spectroscopy, the reaction did not go to completion at room temperature over a prolonged period. Heating the azido nucleoside (91) and triphenyl phosphite in dioxane at reflux for 4 hours afforded the phosphoramidate nucleoside (58) directly with accompanying formation of 2',3',5'-tri-O-acetyladenosine (65). This compound (58) was shown to be identical by the usual criteria to 2',3',5'-tri-O-acetyl-6-N-(diphenylphosphoryl)adenosine prepared earlier (Scheme XIII). The different reactivities exhibited by the two phosphites may

SCHEME XVII


Ac$_2$O
Pyridine
(90)
(91)
(EtO)$_3$P
Dioxane
R.T.
(PhO)$_3$P
Dioxane
reflux
(92)
(58)
0.01N HCl/
H$_2$O
EtOH
(93)
aq.NH$_3$
Dioxane
(94)

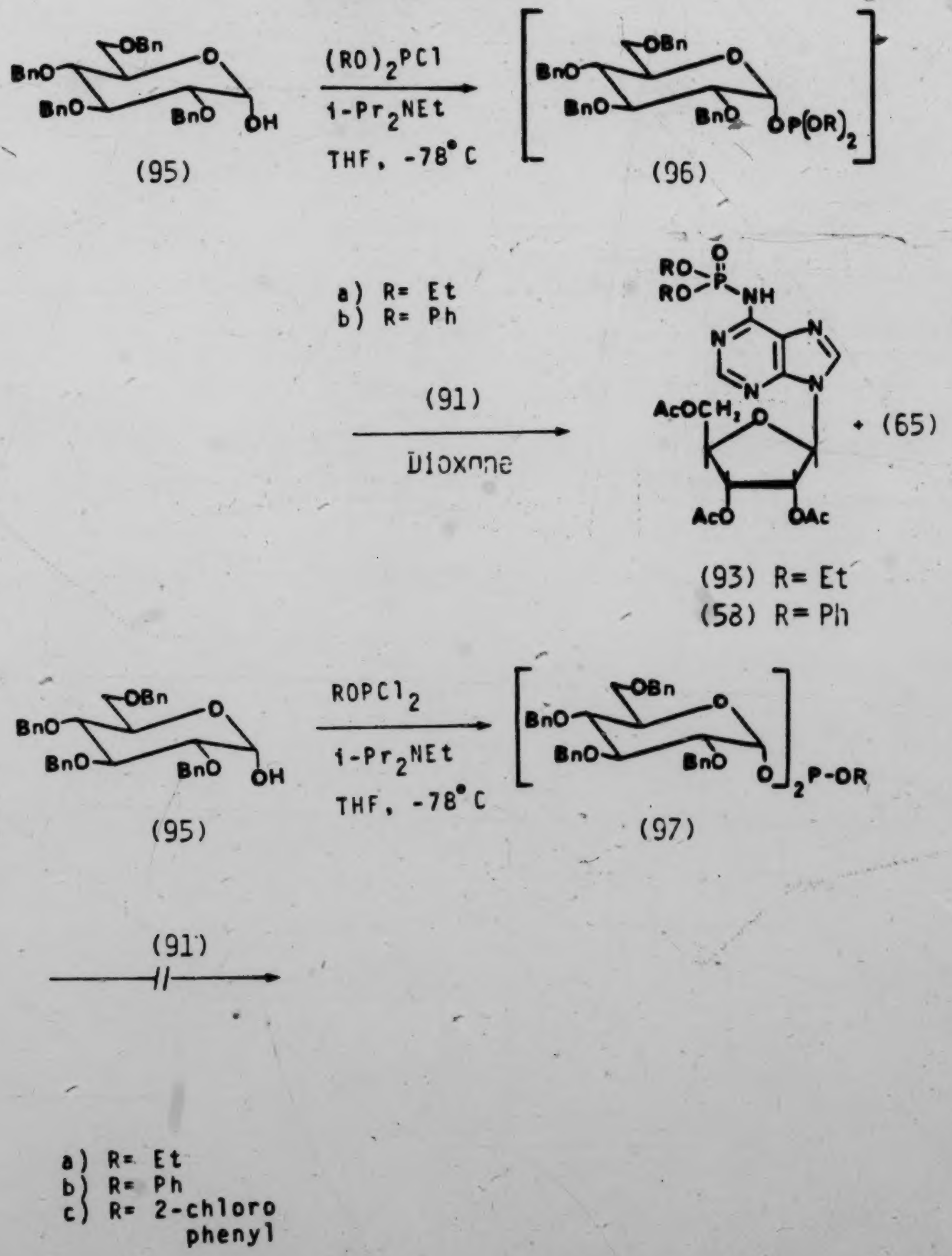
result from the fact that triethyl phosphite is much more nucleophilic than triphenyl phosphite.

We next focussed our attention on coupling the 6-azido nucleoside (91) with sugar phosphites. Treatment of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (95)[114,144] with diethyl phosphorochloridite[145,146] at -78°C gave the mixed phosphite (96). Treatment of (96) with the azido nucleoside (91) in dioxane gave 2',3',5'-tri-*O*-acetyl-6-*N*-(diethylphosphoryl)adenosine (93) by preferential elimination of the sugar (95), which was recovered almost quantitatively. This reaction was also carried out in the presence of lithium chloride (reported to effect selective elimination of ethyl groups[141]). However, the same product (93) was obtained. An analogous reaction sequence beginning with (95) and diphenyl phosphorochloridite gave 2',3',5'-tri-*O*-acetyl-6-*N*-(diphenylphosphoryl)adenosine (58).

We then treated 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (95) with ethyl, phenyl, and 2-chlorophenyl phosphorodichloridites. In all three cases the reactions proceeded quantitatively (as judged by TLC) to give the presumed bis-sugar phosphites (97). These, however, failed to react with the 6-azidopurine nucleoside (91).

Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (98)[147] reacted smoothly with diethyl phosphorochloridite to give

SCHEME XVIII



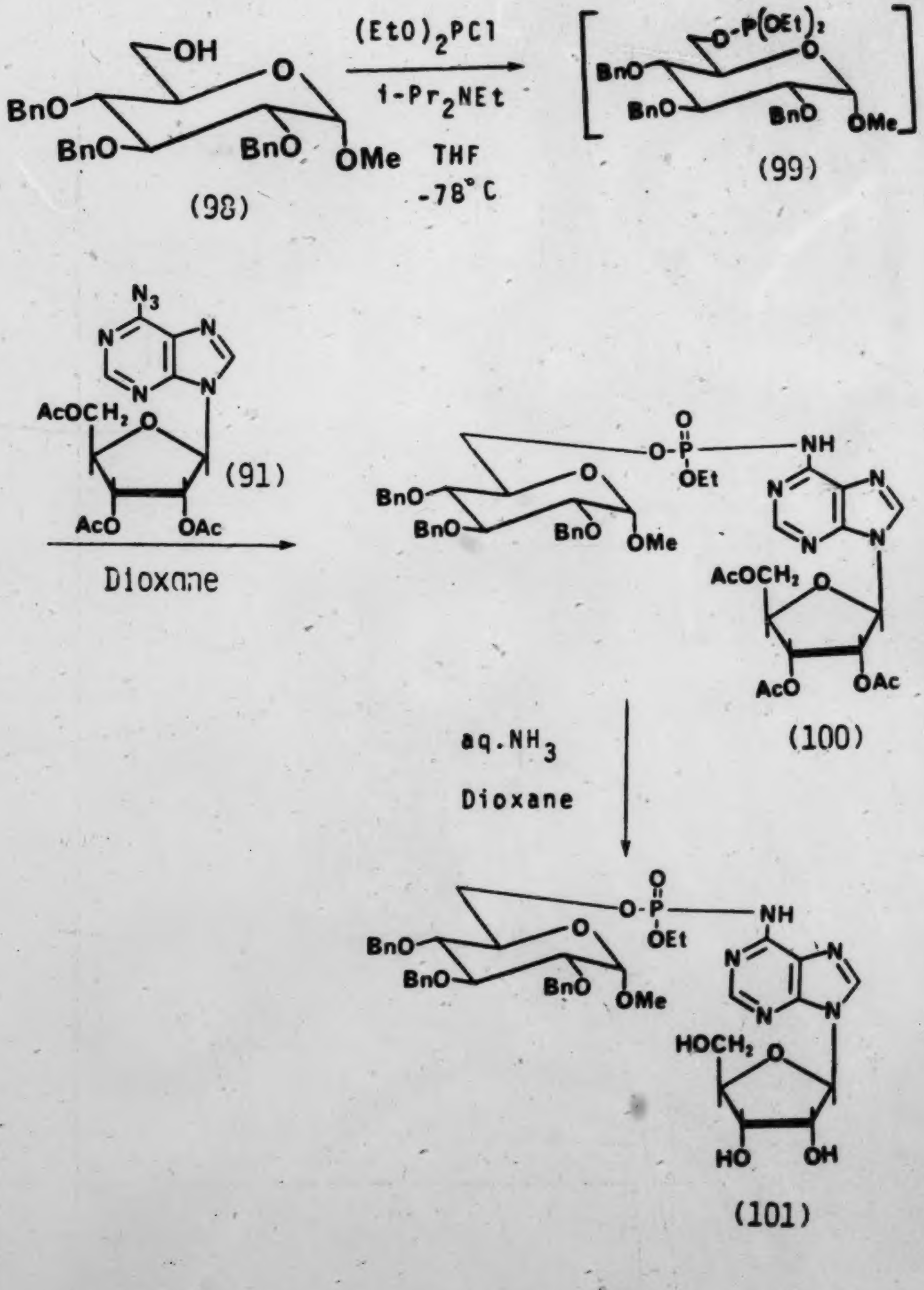
the mixed phosphite intermediate (99) (Scheme XIX). Treatment of (99) with the 6-azido nucleoside (91) gave 2',3',5'-tri-O-acetyl-6-N-[ethyl(methyl 2,3,4-tri-O-benzyl-α-D-glucopyranosid-6-yl)phosphoryl]adenosine (100) in 72% yield after chromatographic purification. It is noteworthy that this coupling occurred with selective elimination of the ethyl group. Subsequent deacetylation afforded 6-N-[ethyl(methyl 2,3,4-tri-O-benzyl-α-D-glucopyranosid-6-yl)phosphoryl]adenosine (101) in 85% yield. The structures of compounds (100) and (101) were confirmed by UV and ^{1}H NMR spectroscopy, FABMS and elemental analyses.

The formation of phosphorimidates (107) and phosphoramidates (108) can be rationalized by any of the three mechanistic pathways depicted in Scheme XX.

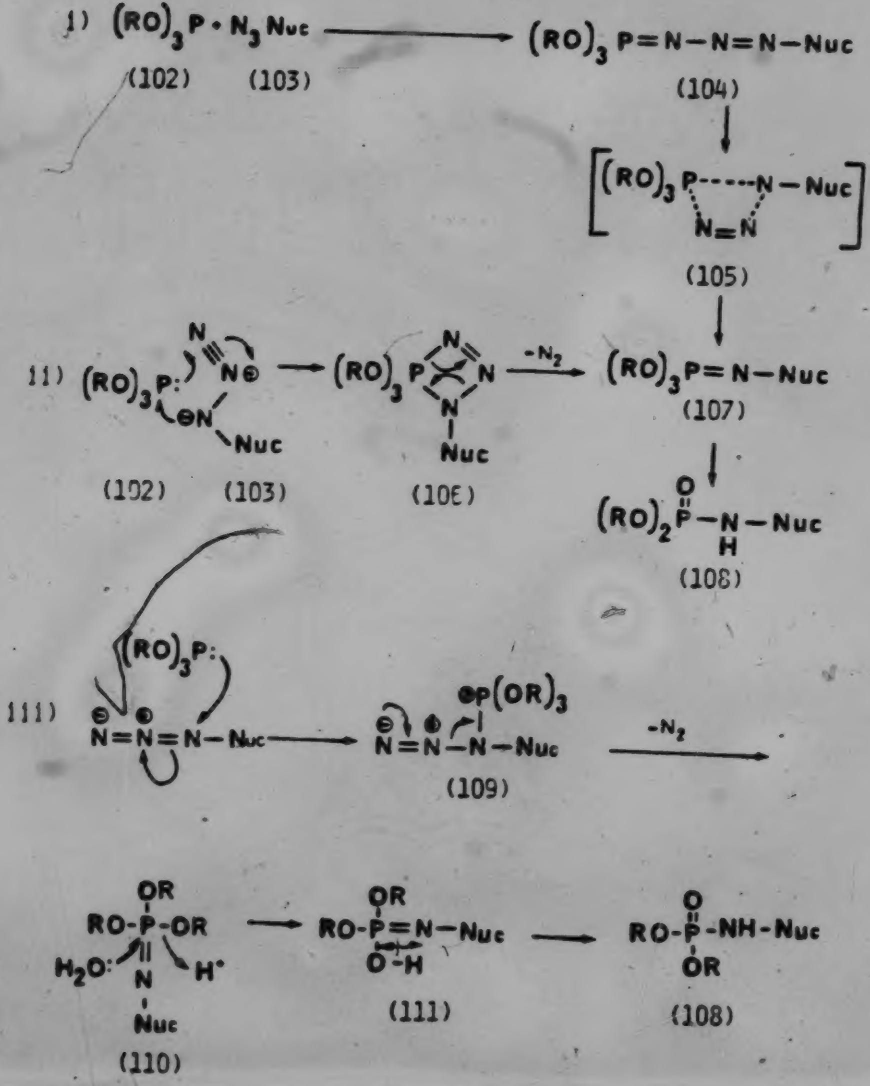
i) In the first case, the phosphite and azide would react to give the phosphazide. This phosphazide then might decompose to give the phosphorimidate by an intramolecular mechanism via a 4-membered transition state (105) (analogous to the mechanism proposed for the Staudinger reaction[148]).

ii) The phosphorimidate might also be formed by initial cycloaddition of phosphite and azide followed by extrusion of N_2.

SCHEME XIX


(EtO)$_2$PCl
i-Pr$_2$NEt
THF
-78° C
(98)
(99)
(91)
Dioxane
(100)
aq.NH$_3$
Dioxane
(101)

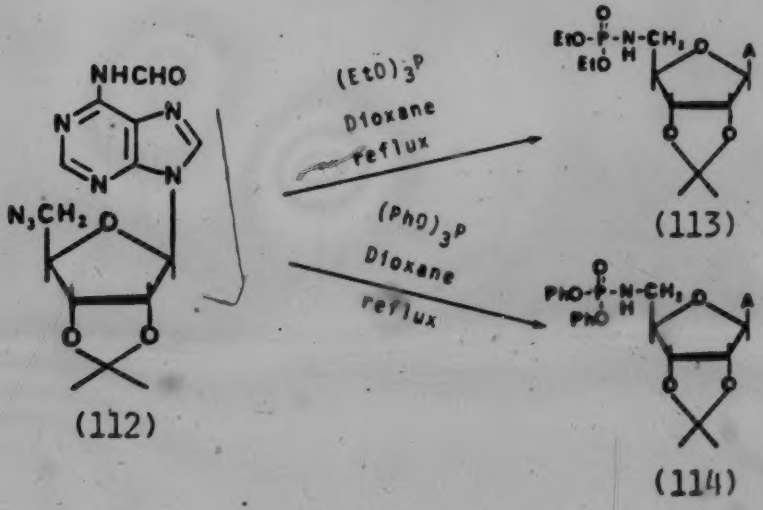
SCHEME XX


i) $(RO)_3P + N_3Nuc$ → $(RO)_3P{=}N{-}N{=}N{-}Nuc$
(102) (103) (104)
$[(RO)_3P\cdots N{-}Nuc,\ N{=}N]$
(105)
ii) $(RO)_3P$: N≡N⊕ ⊖N Nuc
(102) (103)
$(RO)_3P$ N N N Nuc
(106)
$-N_2$
$(RO)_3P{=}N{-}Nuc$
(107)
$(RO)_2P(O){-}NH{-}Nuc$
(108)
iii) $(RO)_3P$:
$N{=}N{=}N{-}Nuc$
$\ominus P(OR)_3$
$N{=}N{-}N{-}Nuc$
(109)
$-N_2$
OR
RO-P-OR
H_2O:
H⁺
N
Nuc
(110)
OR
RO-P=N-Nuc
O-H
(111)
O
RO-P-NH-Nuc
OR
(108)

iii) In the third case, another possible route is shown for the formation of phosphorimidate which could be converted to the phosphoramidate by traces moisture and acid in the reaction mixture.

By means of this phosphite-azide coupling approach, we synthesized two other compounds (113) and (114) (Scheme XXI). 5'-Azido-5'-deoxy-6-N-formyl-2',3'-O-isopropylideneadenosine (112), was obtained via a series of reaction starting with 2',3'-O-isopropylideneadenosine.[149] Reaction of (112) with triethyl and triphenyl phosphite gave compounds (113) and (114) respectively after column chromatography on silica gel. The N-formyl group was removed during chromatography since the silica gel was pretreated with 1,2-dimethoxyethane saturated with ammonia at 0°C.

SCHEME XXI



The phosphite-azide coupling procedure for synthesis of phosphoramidates offers advantages over conventional coupling methods[150-154] that employ amines. The spontaneous reaction of phosphites with azides requires no coupling or activating agents. The reaction conditions are compatible with unprotected hydroxyl and amino functions. The reaction is selective and normally proceeds in high yields.

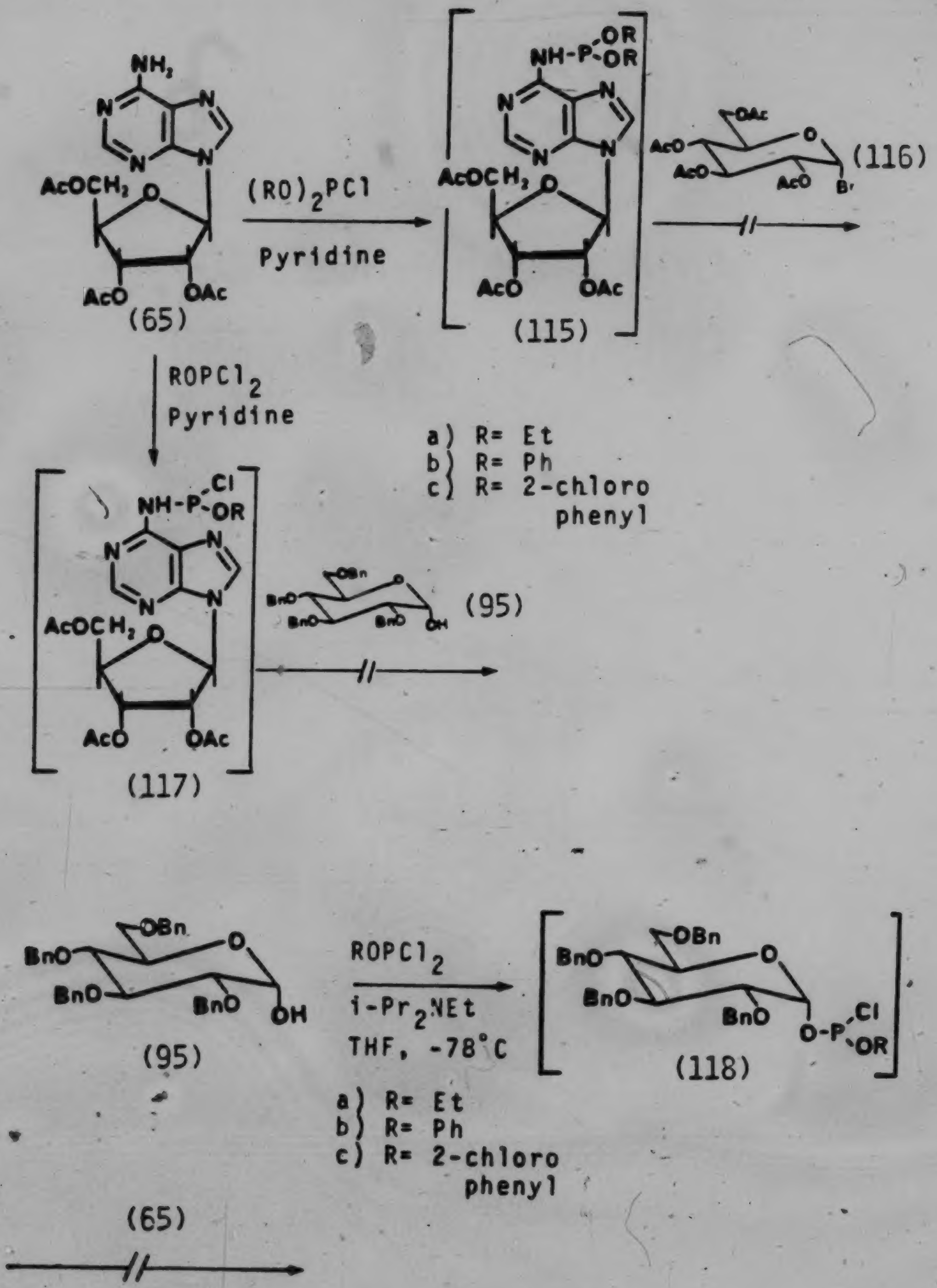
However, since the 6-azido nucleoside (91) failed to react with sugar phosphites (97) and eliminated the sugar in coupling reactions with monoglucosyl phosphites (96) we attempted another approach (Scheme XXII).

Treatment of 2',3',5'-tri-O-acetyladenosine (65) with diethyl, diphenyl or bis-(2-chlorophenyl) phosphorochlordite in pyridine provided the presumed phosphorimidites (115) in virtually quantitative yields (TLC). Attempts to oxidize these products with either MCPBA or iodine-water resulted in quantitative recovery of the original starting material (65). Compounds (115) are unstable on silica gel and give compound (65). Treatment of compounds (115) with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (116)[115] also resulted in quantitative recovery of (65).

Similar treatment of 2',3',5'-tri-O-acetyladenosine (65) with ethyl, phenyl, or 2-chlorophenyl phosphorodichloridites to provide the intermediates (117) followed by reaction with 2,3,4,6-tetra-O-benzyl-α-D-glucopyranose (95) resulted in quantitative recovery of the original starting nucleoside.

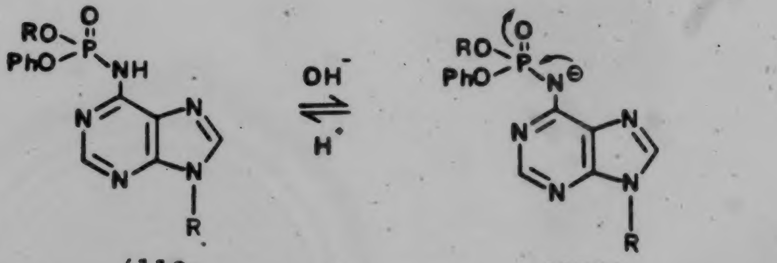
Treatment of 2,3,4,6-tetra-O-benzyl-α-D-glucopyranose (95) with the three phosphorodichloridites to give intermediates (118) followed by reaction with 2',3',5'-tri-O-acetyladenosine (65) also proved disappointing.

SCHEME XXII


(65)
(RO)$_2$PCl
Pyridine
(115)
(116)
ROPCl$_2$
Pyridine
a) R= Et
b) R= Ph
c) R= 2-chloro phenyl
(117)
(95)
(95)
ROPCl$_2$
i-Pr$_2$NEt
THF, -78°C
(118)
a) R= Et
b) R= Ph
c) R= 2-chloro phenyl
(65)

F. UV spectral behaviour of 6-*N*-(phosphoryl)adenosine nucleosides

As can be seen in Tables (1) and (2) there are virtually no shifts in λ_{max} values for the di-*O*-substituted phosphoramidates (entry numbers 1-7 and 9-14) from methanolic to acidic solutions. However, there are significant bathochromic shifts in the basic solutions. The bathochromic shift in basic solution likely results from abstraction of 6-NH proton to give extended conjugation.



(119) (120)

For entry numbers (8) and (15), a bathochromic shift occurs in acidic solution relative to the constant λ_{max} values in methanolic and basic solutions. These λ_{max} values for (8) and (15) parallel those reported for agrocin 84.[63,65] The bathochromic shift in acidic

TABLE. 1

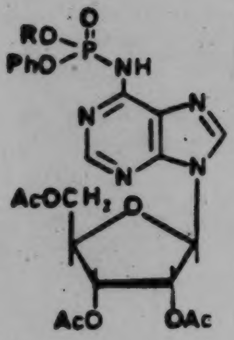
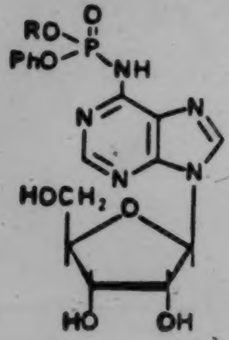
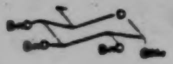
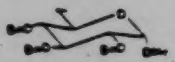


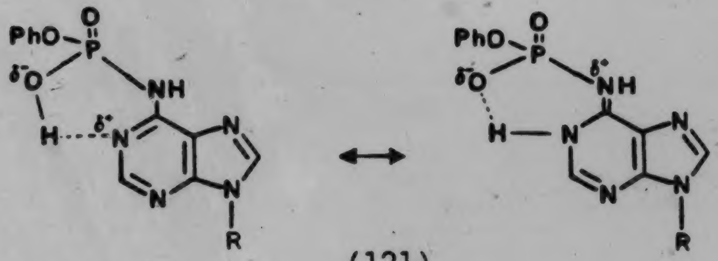
TABLE. 2



Entry Number	R	λ_{max}^{MeOH}	$\lambda_{max}^{H^{+}*}$	$\lambda_{max}^{OH^{-}**}$
1	Ph-	260	260	277
2	Bn-	259	260	276
3	Et-	258	260	276
4	$EtOCH_2CH_2OCH_2CH_2$-	259	260	276
5	Me_2CH-	259	260	276
6	$NCCH_2CH_2$-	261	261	276
7		258	259	277
8	H-	263	267	263
9	Ph-	261	261	276
10	Bn-	261	261	276
11	Et-	260	261	275
12	$EtOCH_2CH_2CH_2CH_2$-	260	260	276
13	Me_2CH-	260	261	276
14		260	261	276
15	H-	263	268	263
	Agrocin 84(1)[63]	264	267.5	
	Agrocin 84(1)[65]	264	268	264

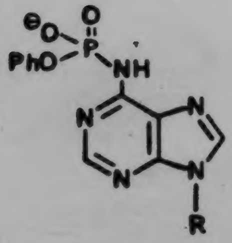
* H^+ =(9 parts 0.1N HCl/H_2O + 1 part methanol)

** OH^- =(9 parts 0.1N $NaOH/H_2O$ + 1 part methanol) solution might result from formation of a conjugated 6-membered proton chelate amidine system as in (121).



(121)

Although a bathochromic shift in basic solution for entry numbers (8) and (15) might have been expected, such a phosphoramidic acid should have a pka <5 and thus would be >99% ionized (122) in the neutral pH region. The negative charge on the free phosphoramidate oxygens would suppress a second anionization of the 6-$NHPO_3Ph^-$ function at higher pH values.



(122)

G. Fast Atom Bombardment Mass Spectrometry (FABMS)

Fast atom bombardment mass spectrometry[156-158] is rapidly becoming a favored method because it can provide spectra of relatively sensitive, non-volatile, ionic, high molecular weight compounds. Reviewers[159,160] of FAB and other ionization techniques[161-165] have had difficulty keeping pace with new discoveries and applications. FAB mass spectra of a large 6-O-methylglucose polysaccharide,[166] bovine insulin,[167] peptides[168,169] and drugs[170,171] exemplify the capabilities of this method.

In electron-impact mass spectra of 6-N-(phosphoryl)adenosine nucleosides and their tri-O-acetyl derivatives, the molecular ion was not observed. Since the molecular weight was the most important item of information for us, we had to use a softer ionization technique. Other ionization methods such as field desorption,[165] californium-252 plasma desorption,[164] secondary ion mass spectrometry with Cs^+[172-174] or Ar^+ ions,[175] laser induced desorption[176] and atmospheric pressure ionization[177] also provide mass spectra of nucleosides and nucleotides. However, the simplicity of FABMS and the ease of conversion of existing mass spectrometers to its use make this approach the most general.[178] A number of examples of FAB mass spectra of nucleotides or derivatives have been reported recently.[156,163,172,179-189]

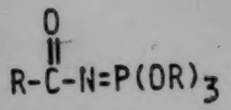
All the compounds were analyzed in the positive ion mode of FAB. These spectra displayed a molecular ion (MH^+) peak and often had an associated sodium (MNa^+), or very rarely a copper-associated (MCu^+) molecular ion peak. We observed that the FAB mass spectra of all the 6-N-(phosphoryl)adenosine nucleosides had (MH^+) peaks. For example compound (81a) with a molecular weight of 513.1413 had a (MH^+) peak at a nominal mass value of m/z 514 and had an associated (MCu^+) peak at m/z 576. Similarly compound (81b) with a molecular weight 451.1256 exhibited a (MH^+) peak at m/z 452 with an associated (MNa^+) peak at m/z 474. Similar results were observed with other 6-N-(phosphoryl)adenosine nucleosides. No chemically consistent fragmentation patterns were observed in this compound series.

FABMS has been developed into as a routine method and has proved to be well suited for the analysis of highly polar compounds. However, one should be aware that the results may be complicated by impact-induced chemical reactions within the matrix.

H. Studies directed toward nucleoside-5'-phosphoramidates

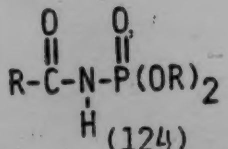
Nucleoside-5'-phosphordiamidates[190,191] derived from ammonia are useful synthetic intermediates. Syntheses of 5'-pyrophosphate and 5'-triphosphate derivatives from purine nucleoside monophosphates were accomplished via phosphoramidate methodology.[192] However, there have been no reports of syntheses of nucleosides with a R-C(O)-NH-P(O)- grouping at the 5'-hydroxyl, analogous to that found in agrocin 84 (1).

N-Acyl phosphorimidates and N-acyl phosphoramidates of the general types (123) and (124) have been recorded.



$R-\overset{O}{\overset{\|}{C}}-N{=}P(OR)_3$

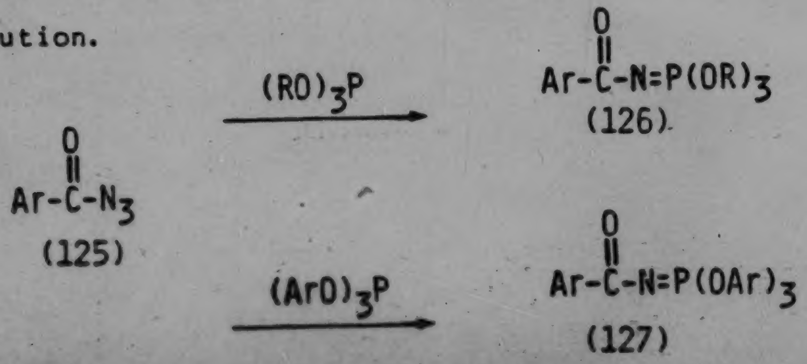
(123)



$R-\overset{O}{\overset{\|}{C}}-\underset{H}{N}-\overset{O}{\overset{\|}{P}}(OR)_2$

(124)

Glidewell[193] has reported syntheses of various N-acyl phosphorimidates by reaction of aromatic carbonyl azides (125) with triaryl or trialkyl phosphites in benzene solution.

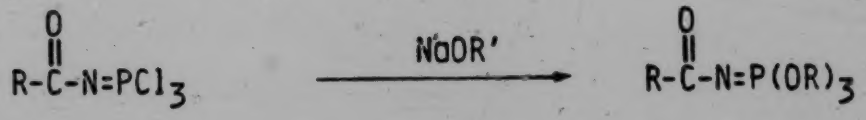


$Ar-\overset{O}{\overset{\|}{C}}-N_3 \xrightarrow{(RO)_3P} Ar-\overset{O}{\overset{\|}{C}}-N{=}P(OR)_3$

$Ar-\overset{O}{\overset{\|}{C}}-N_3 \xrightarrow{(ArO)_3P} Ar-\overset{O}{\overset{\|}{C}}-N{=}P(OAr)_3$

(125) (126) (127)

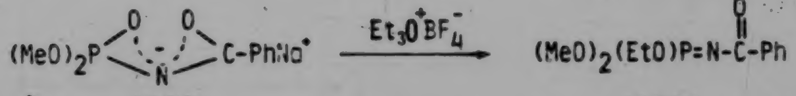
Russian workers have reported syntheses of such phosphorimidates by treatment of trichlorophosphazoacyl intermediates (128) with dry sodium aryloxides in benzene.[194,195]



$$R\text{-}\overset{O}{\overset{\|}{C}}\text{-}N{=}PCl_3 \xrightarrow{NaOR'} R\text{-}\overset{O}{\overset{\|}{C}}\text{-}N{=}P(OR)_3$$

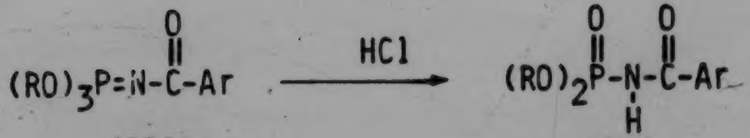
(128) (129)

Recently,[196] the sodium salt of N-benzoyldimethylphosphoramidate (130) was alkylated with triethyloxonium tetrafluoroborate to give compound (131).



(130) (131)

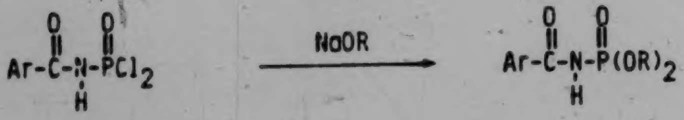
N-Acyl phosphoramidates of the type (124) also have been reported. Glidewell[193] has described conversion of various N-acyl phosphorimidates to N-acyl phosphoramidates by treatment of the former with dry hydrogen chloride in benzene.



$$(RO)_3P{=}N\text{-}\overset{O}{\overset{\|}{C}}\text{-}Ar \xrightarrow{HCl} (RO)_2\overset{O}{\overset{\|}{P}}\text{-}\underset{H}{N}\text{-}\overset{O}{\overset{\|}{C}}\text{-}Ar$$

(126) (132)

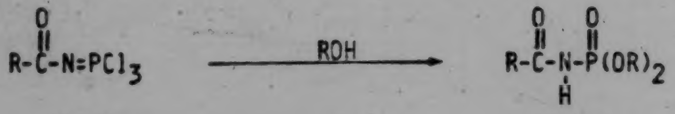
Kirsanov[194] and Derkach[195] have synthesized such N-acyl phosphoramidates by treatment of aroylamidophosphorodichloridates with sodium alkoxides or aryloxides.



$$\mathrm{Ar{-}\overset{O}{\overset{\|}{C}}{-}\underset{H}{N}{-}\overset{O}{\overset{\|}{P}}Cl_2} \xrightarrow{NaOR} \mathrm{Ar{-}\overset{O}{\overset{\|}{C}}{-}\underset{H}{N}{-}\overset{O}{\overset{\|}{P}}(OR)_2}$$

(133) (132)

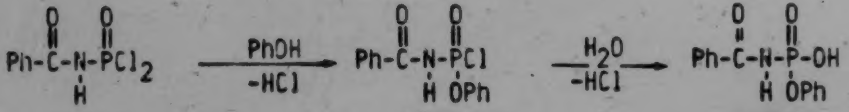
Kirsanov[194] and Steinkopf[197] have reported syntheses of N-acyl phosphoramidates by reaction of alcohols with trichlorophosphazoacyls.



$$\mathrm{R{-}\overset{O}{\overset{\|}{C}}{-}N{=}PCl_3} \xrightarrow{ROH} \mathrm{R{-}\overset{O}{\overset{\|}{C}}{-}\underset{H}{N}{-}\overset{O}{\overset{\|}{P}}(OR)_2}$$

(128) (124)

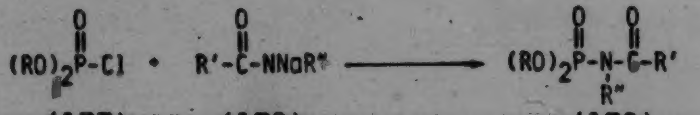
Zioudrou[198] has described the reaction between N-benzoyl phosphorodichloridate[199] with phenol followed by hydrolysis to give phosphoramidate (136).



$$\mathrm{Ph{-}\overset{O}{\overset{\|}{C}}{-}\underset{H}{N}{-}\overset{O}{\overset{\|}{P}}Cl_2} \xrightarrow[-HCl]{PhOH} \mathrm{Ph{-}\overset{O}{\overset{\|}{C}}{-}\underset{H}{N}{-}\underset{OPh}{\overset{O}{\overset{\|}{P}}}Cl} \xrightarrow[-HCl]{H_2O} \mathrm{Ph{-}\overset{O}{\overset{\|}{C}}{-}\underset{H}{N}{-}\underset{OPh}{\overset{O}{\overset{\|}{P}}}{-}OH}$$

(134) (135) (136)

Mizrahi and Modro[200,201] have recently synthesized N-acyl phosphoramidates by treatment of phosphorochloridates with sodium salts of carboxylic amides in toluene.



$$\mathrm{(RO)_2\overset{O}{\overset{\|}{P}}{-}Cl} + \mathrm{R'{-}\overset{O}{\overset{\|}{C}}{-}NNaR''} \longrightarrow \mathrm{(RO)_2\overset{O}{\overset{\|}{P}}{-}\underset{R''}{N}{-}\overset{O}{\overset{\|}{C}}{-}R'}$$

(137) (138) (139)

Our approach to the synthesis of these types of compounds began with the preparation of N-octanoyl-di-O-ethylphosphoramidate (145) (Scheme XXIII). Ethyl octanoate (140) and octanoyl chloride (142) were converted to octanoyl azide (143) by conventional methods.[202,203] Ethyl octanoate was treated with hydrazine and the resulting acylhydrazide (141) treated with nitrous acid to give (143). Treatment of (142) with sodium azide in acetone gave octanoyl azide (143) directly. In both cases quantitative conversion to the acyl azide was assumed.

A solution of octanoyl azide (143) in ether was heated at reflux with triethyl phosphite in dioxane to give N-octanoyl-tri-O-ethylphosphorimidate (144) in almost quantitative yield. Treatment of the latter compound (144) under acidic conditions gave the desired N-octanoyl-di-O-ethylphosphoramidate (145).

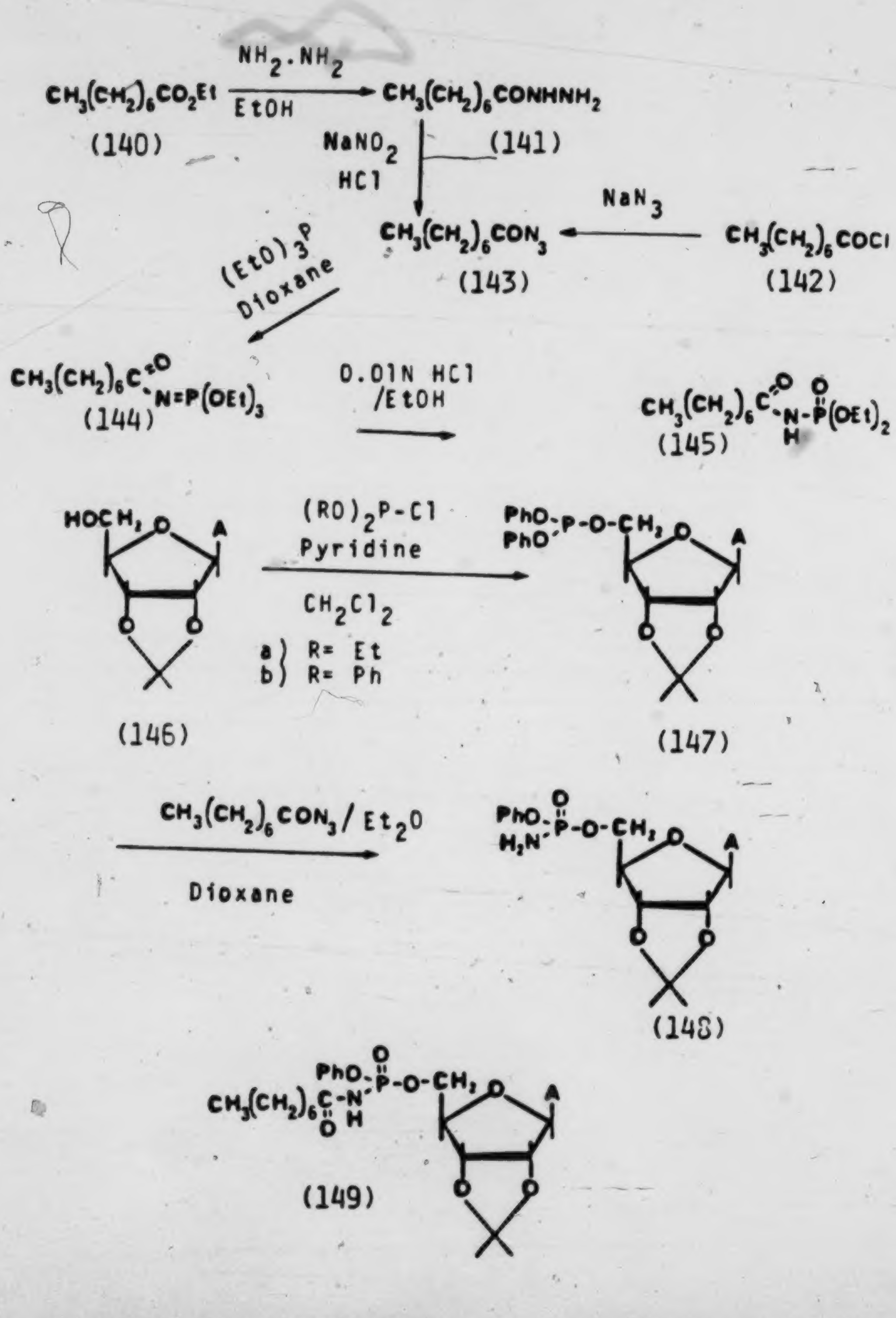
In order to proceed by analogy with this phosphite-acyl azide reaction to give (145) we attempted to prepare 5'-O-(diethylphosphityl)-2',3'-O-isopropylideneadenosine (147a) by reaction of 2',3'-O-isopropylideneadenosine (146). However, attempted isolation of compound (147a) was not successful since the presumed product (TLC) decomposed to give back starting nucleoside (146). Diphenyl phosphorochloridite[204] reacted smoothly with (146) to give

5'-O-(diphenylphosphityl)-2',3'-O-isopropylideneadenosine (147b).

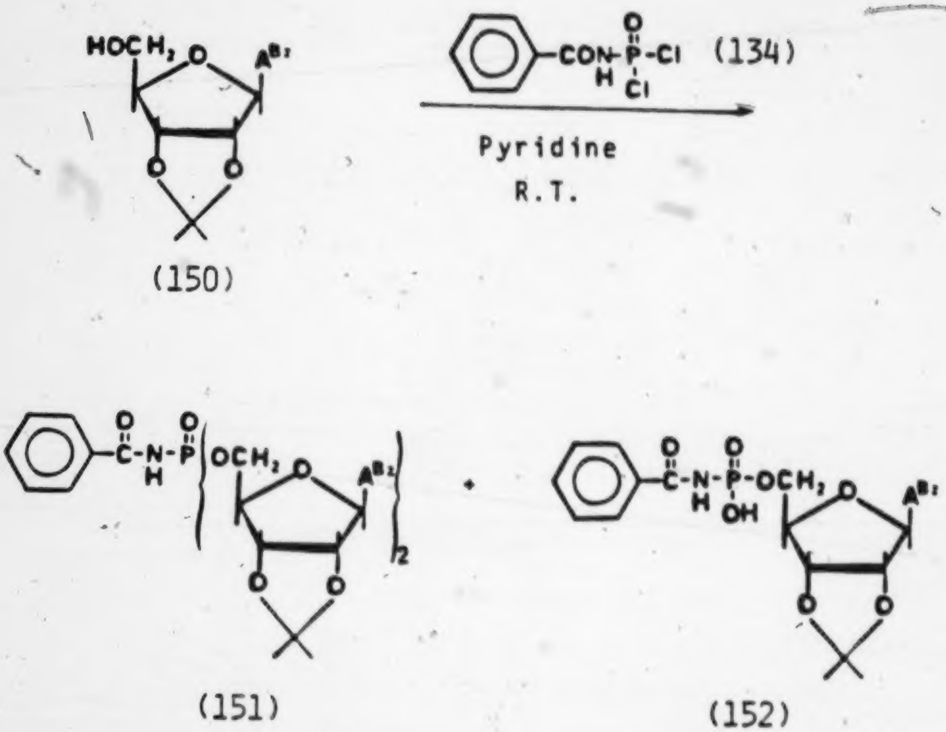
Reaction of (147b) with octanoyl azide (143) in refluxing dioxane yielded 5'-O-(phenylphosphoramidatyl)-2',3'-O-isopropylideneadenosine (148) and (146) in a ratio of 65:35. The ^{1}H NMR spectra showed the absence of octanoyl groups. The reaction was conducted under various conditions, but the desired compound (149) was not obtained. Compound (148) was obtained in good yield when the ratio of azide (143) to phosphite (147b) was 5:1.

However, we did succeed in making a compound containing a -C(O)-NH-P(O)-linkage at the 5'OH of 6-N-benzoyl-2',3'-O-isopropylideneadenosine (150) (obtained from 2',3'-O-isopropylideneadenosine (146) according to a procedure described by Jones[205]). Thus N-benzoyl phosphorodichloridate (134)[199] reacted smoothly with (150) in pyridine (Scheme XXIV) to give the bis-substituted product (151) and 6-N-benzoyl-5'-O-[(N-benzoyl)-phosphoramidatyl]-2',3'-isopropylideneadenosine (152). Formation of compound (152) was indicated by the presence of a corresponding MH^+ peak in its FAB mass spectrum and also by its ^{1}H NMR spectrum. However, attempts to obtain the pure compound (152) proved unsuccessful. (The ^{1}H NMR spectrum of (152) indicated the presence of impurities).

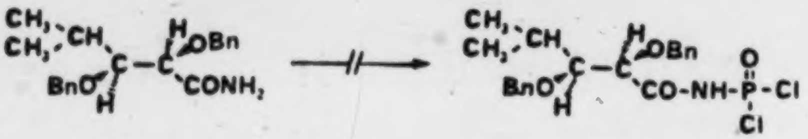
SCHEME XXIII



SCHEME XXIV


HOCH₂
O
A^{Bz}
O
O
CON-P-Cl
H
O
Cl
(134)
Pyridine
R.T.
C-N-P
OCH₂
2
OH
+
(150)
(151)
(152)

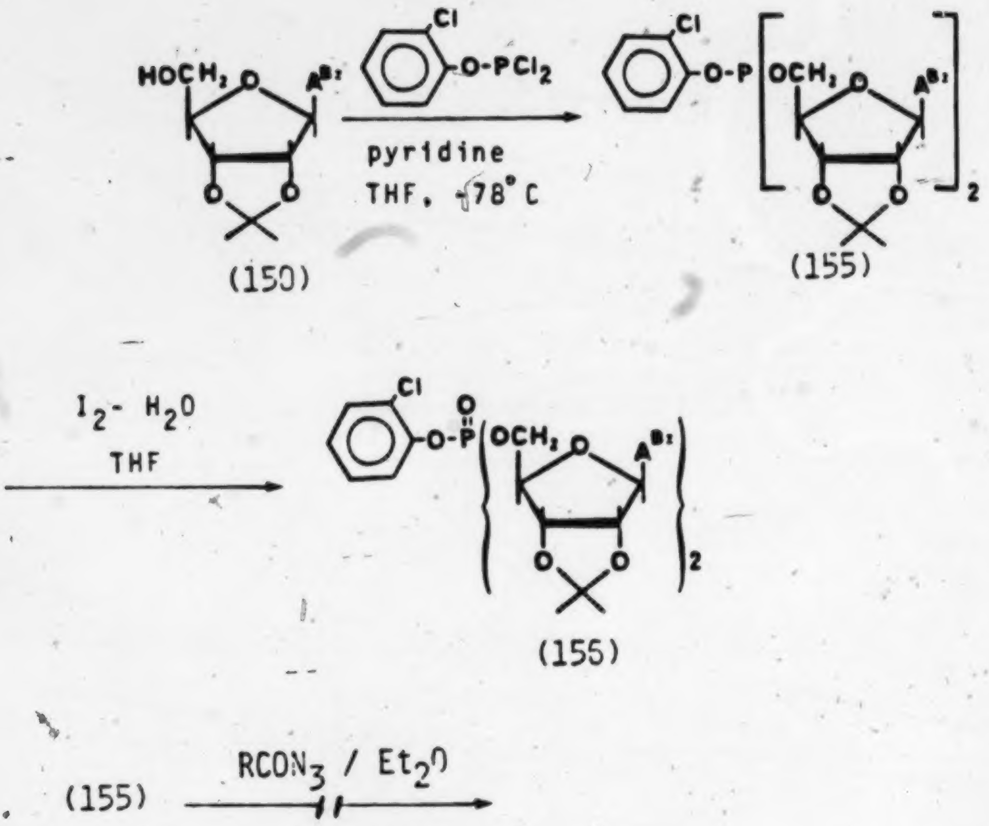
Using the approach of Titherly and Worrall[199] we attempted phosphorylation of DL-*threo*-2,3-di-*O*-benzyl-4-methylpentanamide (153) with phosphorus pentachloride. [Compound (153) was obtained from DL-*threo*-2,3-dihydroxy-4-methylpentanamide (7') by benzylation.]



(153) (154)

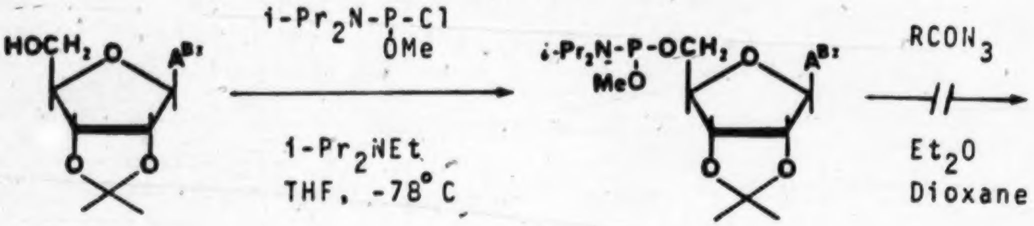
This reaction failed to give compound (154). Perhaps the phosphorylation product is unstable and decomposes readily. Only amides with aromatic (or aliphatic with electron withdrawing groups on the α-carbon atom) acyl groups are known to form stable phosphoramidodichloridates.[206] In another attempt (Scheme XXV) compound (150) was reacted with 2-chlorophenyl phosphorodichloridite to give the mixed phosphite (155). Formation of this compound (155) was confirmed by its oxidation with iodine[207,208] to give the corresponding phosphotriester (156). Compound (155), however, failed to react in the desired manner with octanoyl azide (143) in a refluxing solution of dioxane-ether. Phosphite (155) is quite stable in a refluxing solution of dioxane-ether, but is converted to starting compound (150) in the presence of the acyl azide (143).

SCHEME XXV



R = $CH_3-(CH_2)_5-$

6-N-Benzoyl-2',3'-O-isopropylideneadenosine (150) was treated with "N,N-diisopropylmethylphosphonamidic chloride"* to give the intermediate (157). Compound (157) decomposed to starting material (150) on silica gel. Treatment of compound (157) in situ with octanoyl or hexanoyl azides also resulted in quantitative reisolation of starting material (150).



(150)

(157)

a) R= $CH_3-(CH_2)_6-$

b) R= $CH_3-(CH_2)_4-$

---

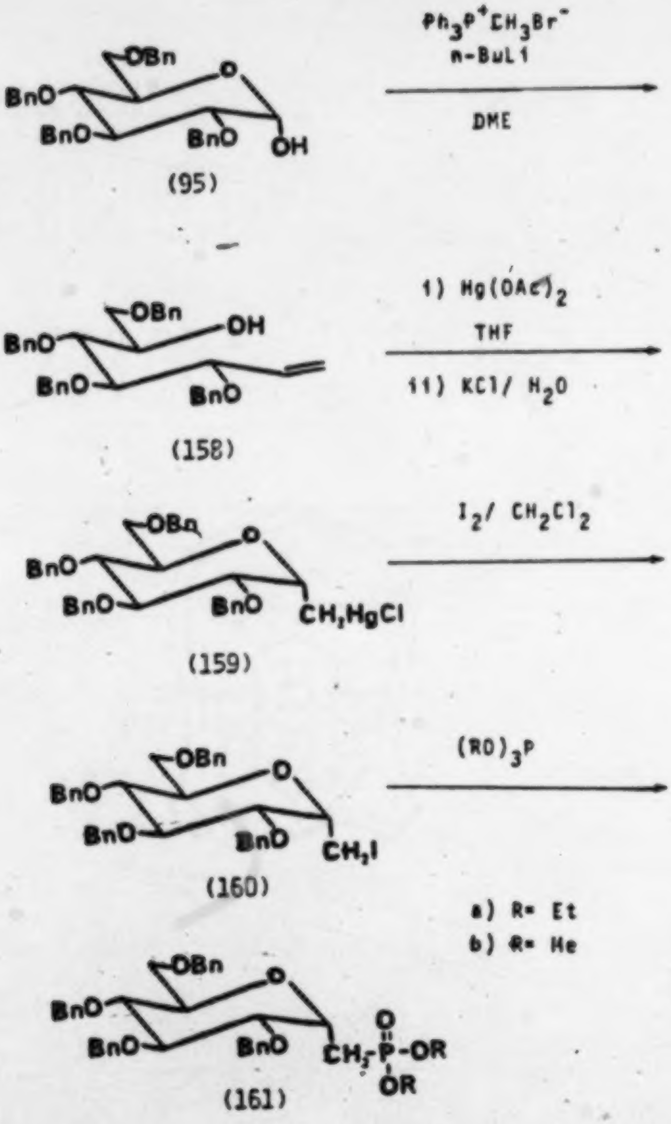
* Aldrich Chemical Co. named reagent.

I. Syntheses of Sugar phosphonates

Phosphonate analogues of amino acids as well as of naturally occurring phosphoric esters such as phosphoglycerides, nucleotides, and sugar phosphates are of interest as potential inhibitors of biological processes.[209] In the carbohydrate field, a number of examples have been made, but analogues in which the methylphosphonate function is linked to the anomeric carbon have been reported only recently.[210-216] Since attempts to link the glycosyl moiety through the anomeric OH had failed, we attempted to synthesize carbon-linked phosphoramidates.

When 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (95) was subjected to the Wittig reaction with methylene-triphenylphosphorane, the unsaturated derivative (158) was obtained.[217] Stereoselective mercuriocyclization and treatment with potassium chloride afforded derivative (159).[218] Treatment of compound (159) with iodine in dichloromethane gave the corresponding iodomethyl derivative (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-methyl iodide (160) in 85% yield after chromatography. The iodo derivative (160) was subjected to the Arbuzov reaction with triethyl phosphite to give the phosphonic ester (161a) in 94% yield. Synthesis of this compound via the bromomethyl derivative has been reported by Nicotra *et al.*[211] in 30% overall yield from the mercurio derivative. Treatment of the iodo derivative (160) with

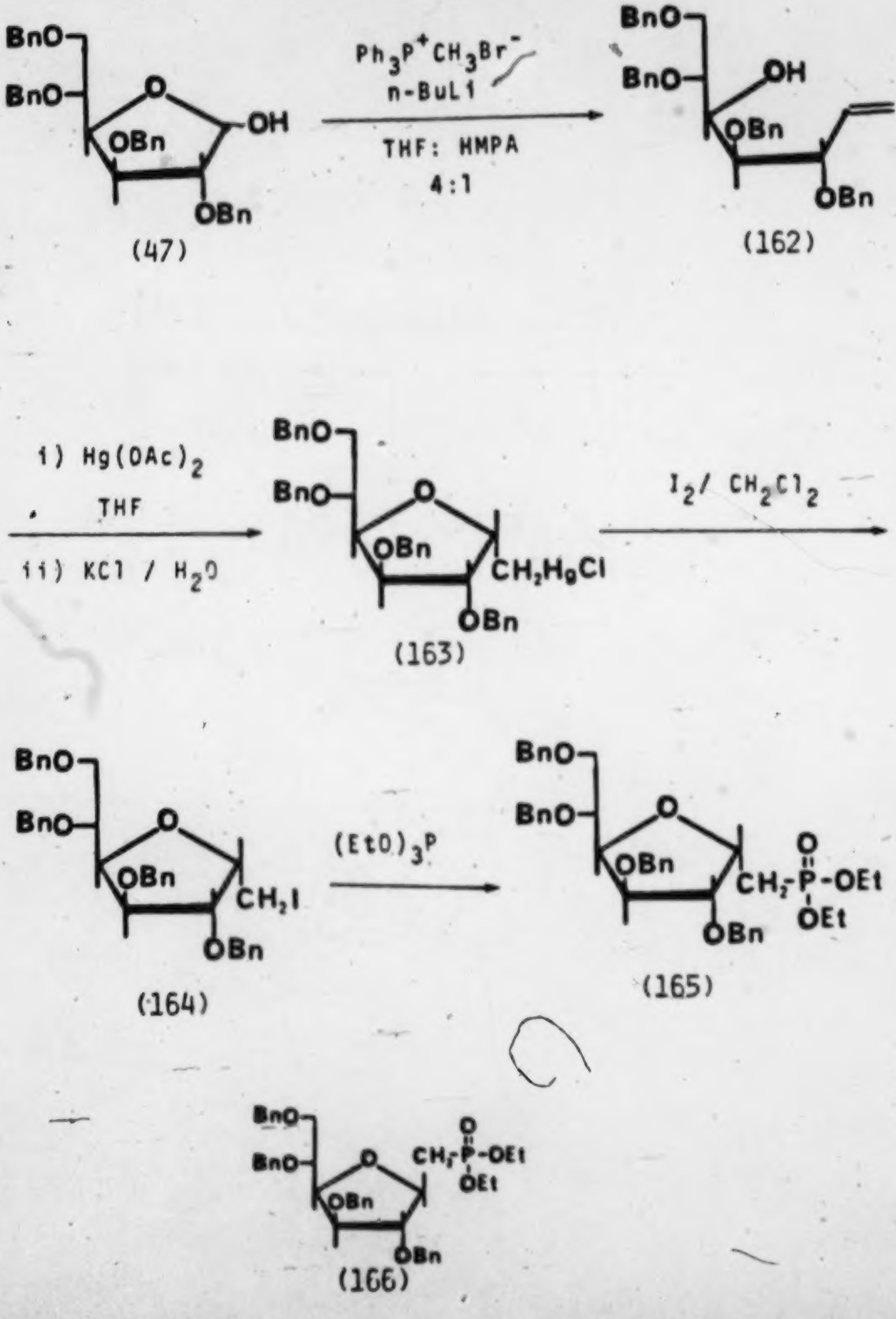
SCHEME XXVI



trimethyl phosphite gave a 47% yield of dimethyl (2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)methylphosphonate (161b).

No reaction was observed when 2,3,5,6-tetra-O-benzyl-D-glucofuranose (47) was subjected [illegible] the Wittig reaction under identical conditions as above$^{[illegible]17}$. Nicotra et al.^{216} have recently reported the successful Wittig reaction of 2,3-O-isopropylidene-5-O-trityl-D-ribofuranose with methylenetriphenylphosphorane using THF-HMPA (4:1) as solvent. Our furanose derivative (47) underwent a similar Wittig reaction in THF-HMPA (4:1) to give 3,4,6,7-tetra-O-benzyl-1,2-dideoxy-D-glucohept-1-enitol (162) in 74% yield. Mercuriocyclization of this unsaturated derivative afforded predominantly^{219} the 1,2-cis product and (2,3,5,6-tetra-O-benzyl-α-D-glucofuranosyl)methylmercury chloride (163) was obtained in 78% yield. Treatment of compound (163) with iodine followed by Arbuzov reaction with triethyl phosphite gave diethyl (2,3,5,6-tetra-O-benzyl-α-D-glucofuranosyl)methylphosphonate (165). [The cis-1,2-configuration of this compound (165) was determined on the basis of the small ^{13}C-^{1}H coupling constant $J_{C1'-H2}$ = 1.5 Hz]. The ^{31}P NMR spectrum of this compound showed an intense peak at +28.92 ppm (upfield from H_3PO_4) and a small peak at +27.21 ppm, which can be attributed to the presence of a small amount of the trans-

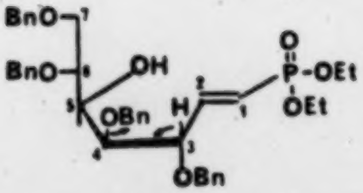
SCHEME XXVII



1,2-anomer (166). The anomeric ratio of (165)/(166) was >95:5 as determined by the integration of the intensities of the two peaks in ^{31}P NMR spectrum. Attempted chromatographic separation of these anomers failed.

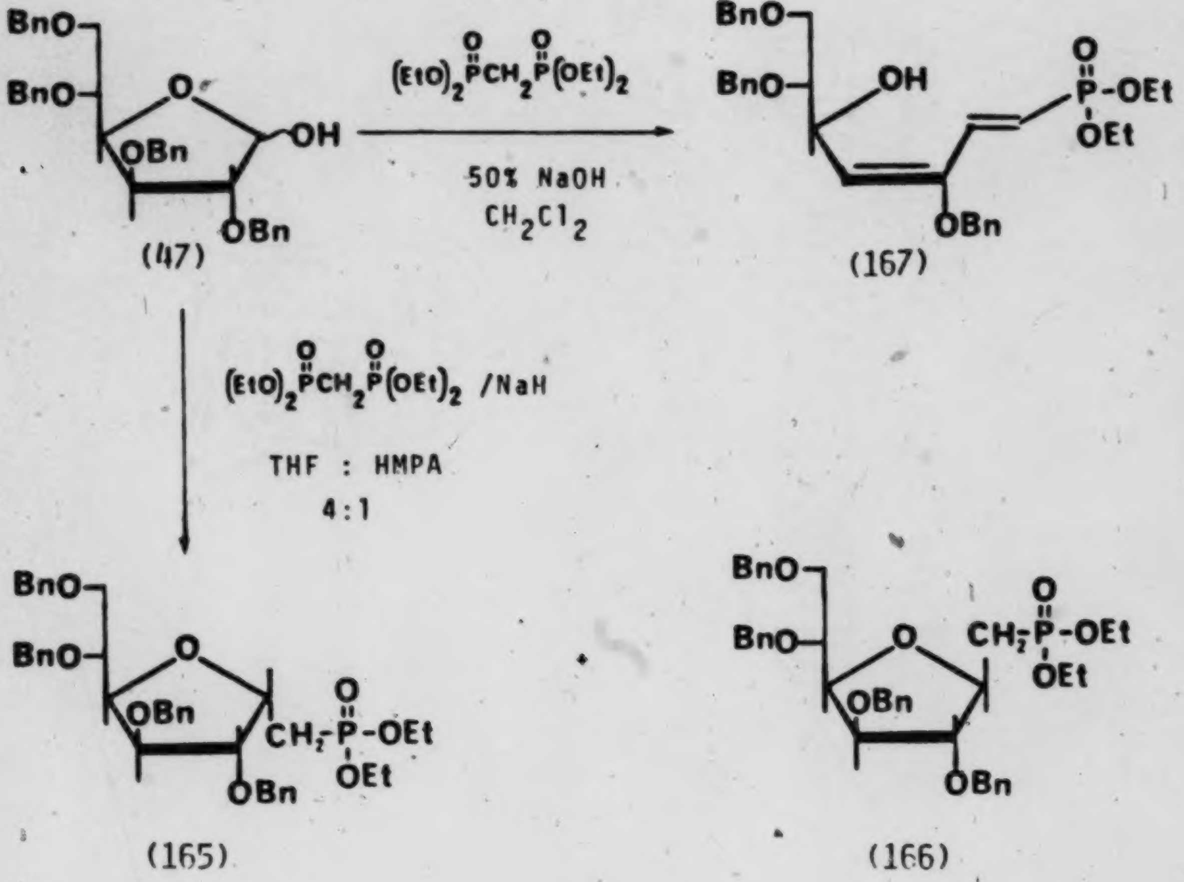
Meyer *et al*.[215] reported the condensation of 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribose with tetramethyl methylenebisphosphonate. A mixture of 2,5-anhydro-1-deoxy-1-(diethoxyphosphinyl)-2,3-*O*-isopropylidene-5-*O*-trityl-D-altritol and allitol was obtained in a ratio of 3:2.

We employed the analogous reaction of tetraethyl methylenebisphosphonate with 2,3,5,6-tetra-*O*-benzyl-D-glucofuranose (47). However, the unsaturated derivative (167) (Scheme XXVIII) was obtained. The formation of compound (167) can be rationalized on the basis that compound (168) is formed in the initial reaction.



(168)

SCHEME XXVIII


BnO
BnO
O
OBn
OH
OBn
(47)
$(EtO)_2\overset{O}{\overset{\|}{P}}CH_2\overset{O}{\overset{\|}{P}}(OEt)_2$
50% NaOH
CH_2Cl_2
BnO
BnO
OH
P-OEt
OEt
OBn
(167)
$(EtO)_2\overset{O}{\overset{\|}{P}}CH_2\overset{O}{\overset{\|}{P}}(OEt)_2$ /NaH
THF : HMPA
4:1
BnO
BnO
O
OBn
CH_2-P-OEt
OEt
OBn
(165)
BnO
BnO
O
CH_2-P-OEt
OEt
OBn
OBn
(166)

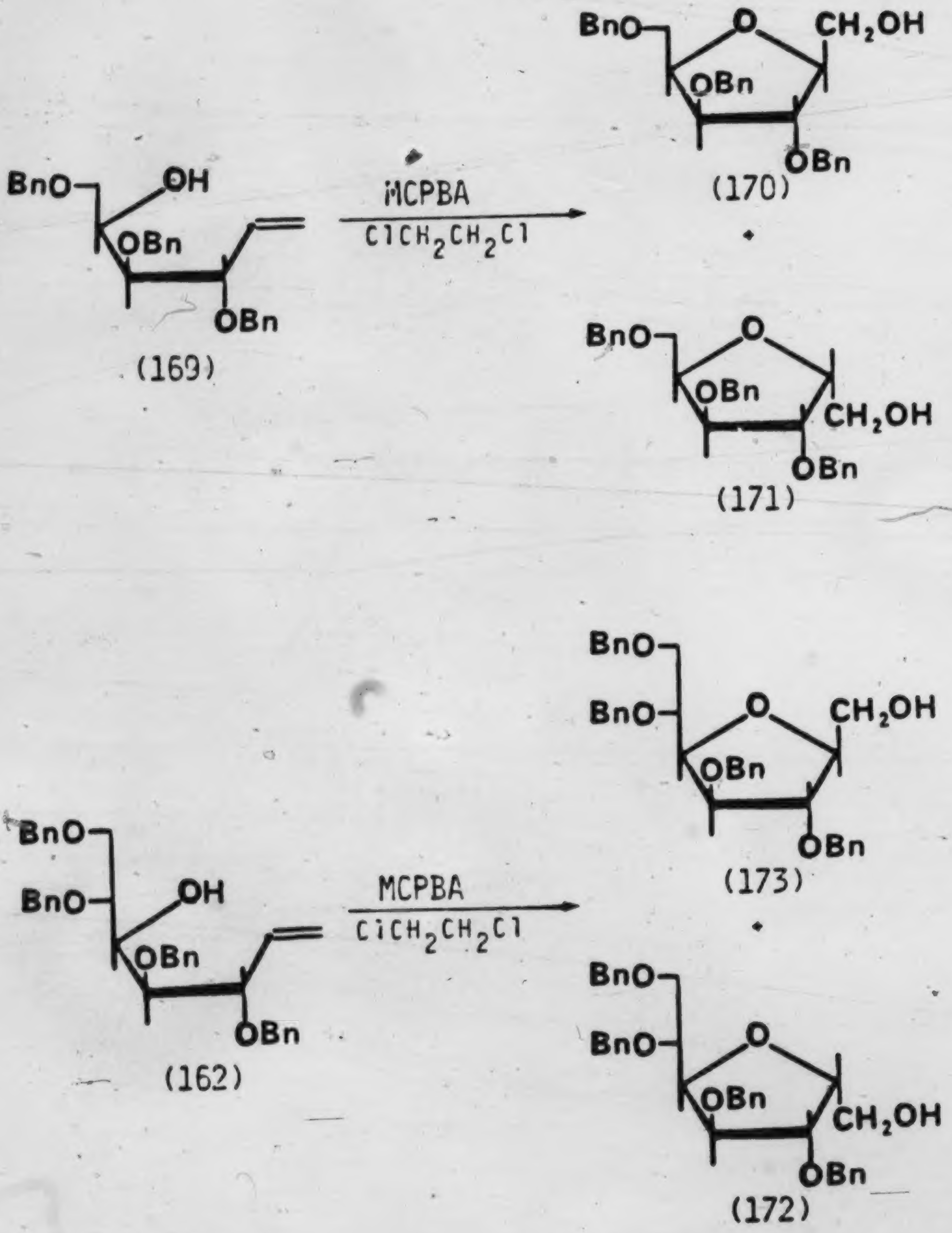
Under the strongly basic conditions, the H-3 proton is abstracted, resulting in syn-elimination of the C-4-benzyloxy group to give the conjugated diene (167).

Treatment of compound (47) with tetraethyl methylenebisphosphonate[220] and sodium hydride in THF-HMPA (4:1) gave a mixture of the desired α (165) and β (166) anomers in a ratio of 2:3 (determined by integration of ^{31}P NMR spectrum). Attempted selective deprotection of the ethyl groups from these glycosylmethylphosphonates had failed. Hence, syntheses of carbon-linked phosphoramidates was abandoned.

Reitz and coworkers[221] have recently reported the cyclization of hydroxyalkene (169) to alcohols (170) and (171) with MCPBA (Scheme XXIX). Our analogous cyclization of the unsaturated derivative (162) gave a mixture of α (172) and β (173) alcohols in ~1:1 ratio. Separation of these two alcohols was achieved by column chromatography on silica gel. Neither of the alcohols reacted satisfactorily with diethyl phosphorochloridite to give the desired mixed phosphite.

Treatment of 2',3',5'-tri-*O*-acetyladenosine (65) with phenyl phosphorodichloridate followed by reaction with either of the isomeric alcohols (172 or 173) in the presence of silver carbonate led to decomposition of the alcohols.

SCHEME XXIX


BnO
OH
OBn
OBn
(169)
MCPBA
$ClCH_2CH_2Cl$
BnO
O
CH_2OH
OBn
OBn
(170)
+
BnO
O
OBn
CH_2OH
OBn
(171)
BnO
BnO
OH
OBn
OBn
(162)
MCPBA
$ClCH_2CH_2Cl$
BnO
BnO
O
CH_2OH
OBn
OBn
(173)
+
BnO
BnO
O
OBn
CH_2OH
OBn
(172)

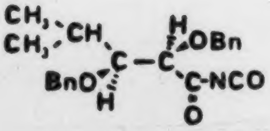
J. Syntheses of compounds analogous to agrocin 84

After having synthesized the three component units of agrocin 84 and having studied a number of model reactions for the syntheses of 6-*N*-(phosphoryl)adenosine nucleosides and towards nucleosides containing a R-C(O)-NH-P(O) group at the 5'-OH, we focussed some attention on compounds which could be analogues of agrocin 84.

Octanoyl isocyanate (175) was chosen as a precursor to mimic the side chain function at the 5'-OH of the "core nucleoside" (3) of agrocin 84 (1). Octanoyl isocyanate (175) was prepared from octanoyl chloride and silver isocyanate as described for the preparation of some acetyl isocyanates.[222] Treatment of 6-*N*-benzoyl-2',3'-*O*-isopropylideneadenosine (150) with octanoyl isocyanate in dichloromethane afforded 6-*N*-benzoyl-2',3'-*O*-isopropylidene-5'-*O*-[(*N*-octanoyl)carbamoyl]adenosine (176) in virtually quantitative yield. The isopropylidene group was removed from (176) by trifluoroacetic acid to give 6-*N*-benzoyl-5'-*O*-[(*N*-octanoyl)carbamoyl]adenosine in 96% yield. Similarly, reaction of 6-*N*-benzoyl-9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenosine (178) [This compound was obtained from 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3) by a procedure described by Jones *et al.*[205]] with octanoyl isocyanate provided 6-*N*-benzoyl-5'-*O*-[(*N*-

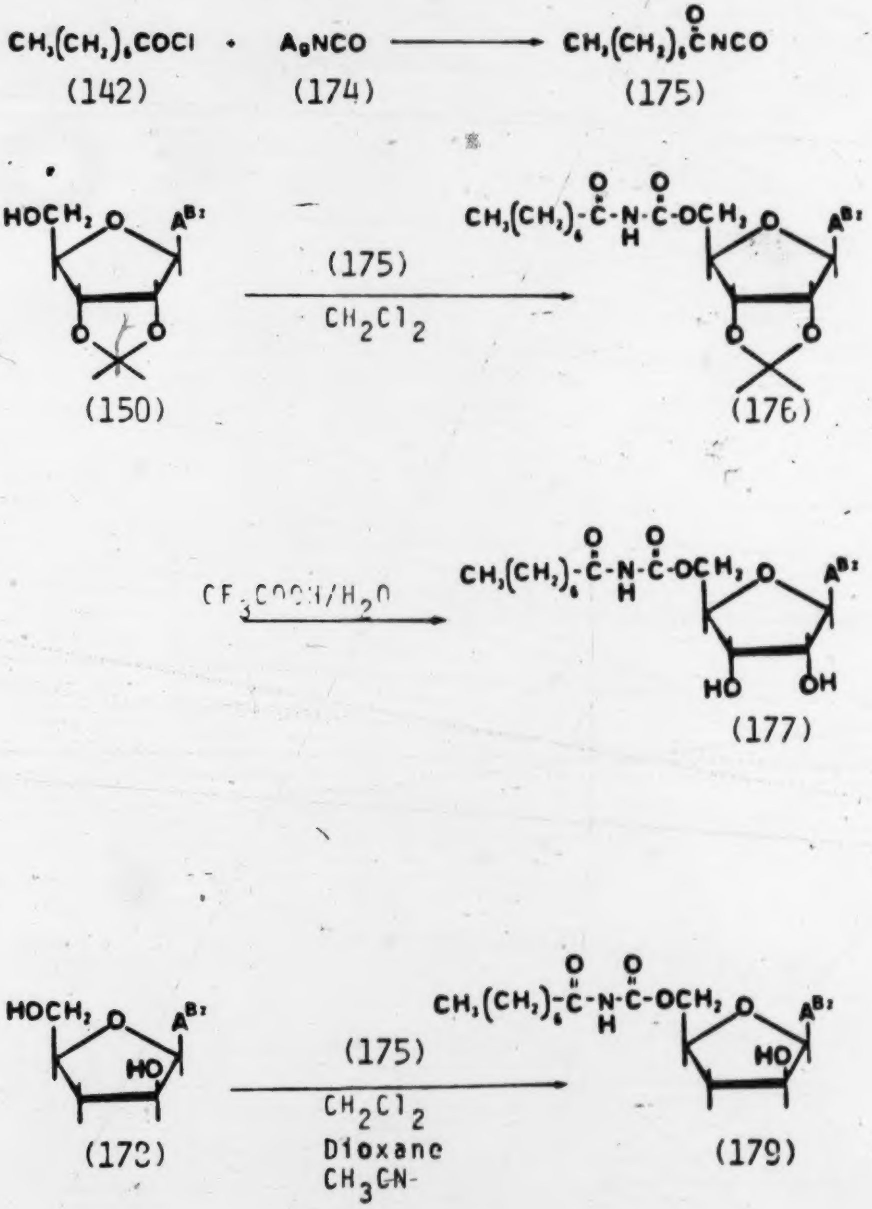
octanoyl)-carbamoyl]-9-(3-deoxy-β-*D*-*threo*-pentofuranosyl)adenine (179) in ~60% yield.

5'-Azido-5'-deoxy-6-*N*-formyl-2',3'-*O*-isopropylideneadenosine (112)[149] was hydrogenolyzed over 5% palladium/charcoal to give a mixture of 5'-amino-5'-deoxy-6-*N*-formyl-2',3'-*O*-isopropylideneadenosine (180) and 5'-amino-5'-deoxy-2',3'-*O*-isopropylideneadenosine (181) in nearly equal amounts. These compounds were separated by chromatography on silica gel. Compound (180) was treated with octanoyl isocyanate (175) to give 5'-deoxy-5'-*N*-[(*N*-octanoyl)carbamoyl]amino-6-*N*-formyl-2',3'-*O*-isopropylideneadenosine (182) in 84% yield. Removal of the isopropylidene group from compound (182) by trifluoroacetic acid gave 5'-deoxy-5'-*N*-[(*N*-octanoyl)carbamoyl]-amino-6-*N*-formyladenosine (183). Currently we are trying to obtain compound (184) from (183) by deprotection of the 6-*N*-formyl group. We are also investigating methods to obtain the agrocin 84 related isocyanate (185) to provide a closer analogue.

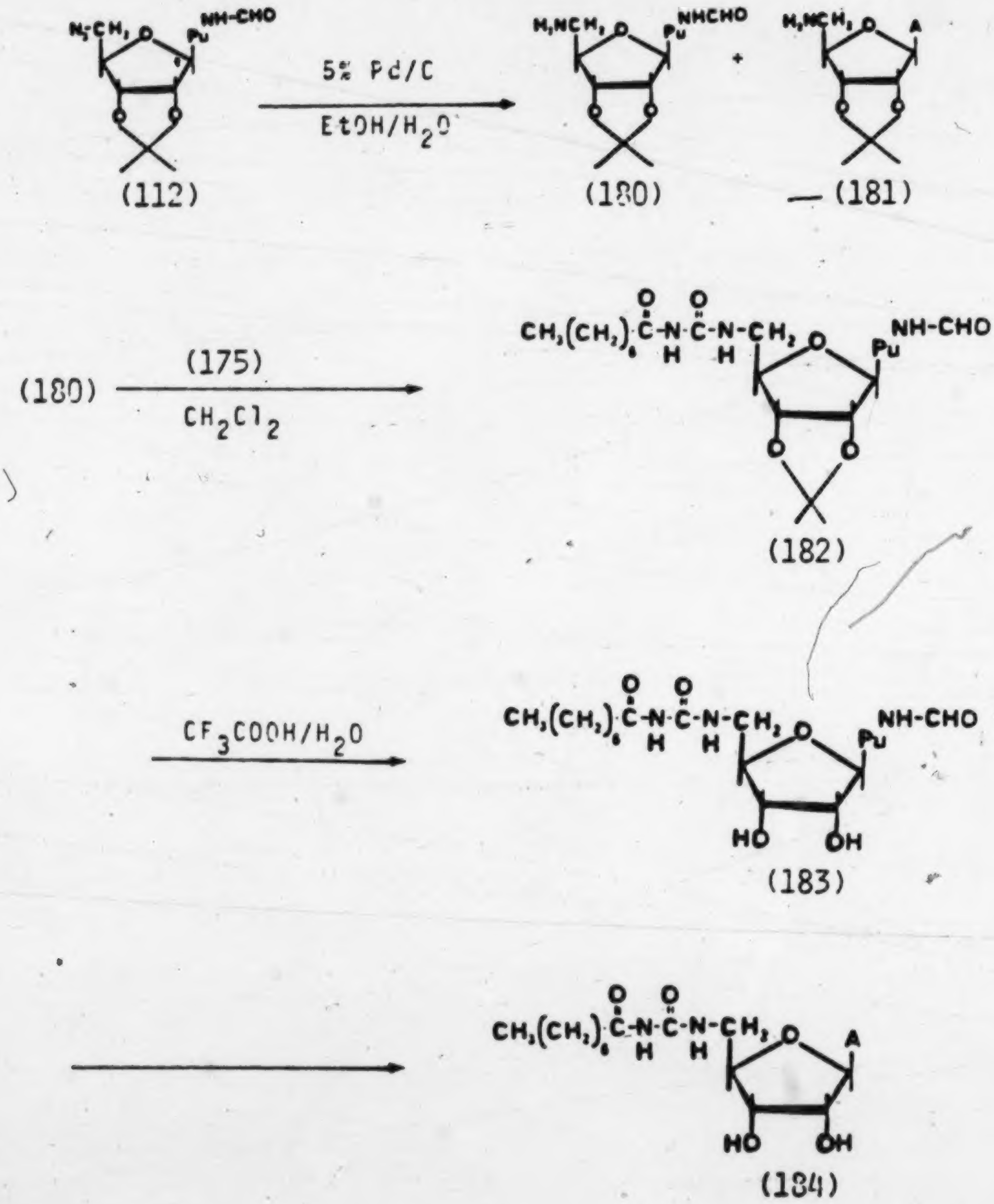


(185)

SCHEME XXX


CH3(CH2)6COCl + AgNCO → CH3(CH2)6CNCO
(142)
(174)
(175)
HOCH2
ABz
(175)
CH2Cl2
CH3(CH2)6-C-N-C-OCH2
H
(150)
(176)
CF3COOH/H2O
HO
OH
(177)
HO
(175)
CH2Cl2
Dioxane
CH3CN-
(178)
(179)

SCHEME XXXI



H_2NCH_2
NH-CHO
Pu
(112)
5% Pd/C
$EtOH/H_2O$
H_2NCH_2
NHCHO
Pu
(180)
+
H_2NCH_2
A
(181)
(180)
(175)
CH_2Cl_2
$CH_3(CH_2)_6C(O)-NH-C(O)-NH-CH_2$
NH-CHO
Pu
(182)
CF_3COOH/H_2O
$CH_3(CH_2)_6C(O)-NH-C(O)-NH-CH_2$
NH-CHO
Pu
HO
OH
(183)
$CH_3(CH_2)_6C(O)-NH-C(O)-NH-CH_2$
A
HO
OH
(184)

EXPERIMENTAL

A. General Procedures

Melting points were determined on a Reichert microstage apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on Varian HA-100, Bruker WH-200, Bruker AM-300 or Bruker WH-400 spectrometers operating in the FT mode, with tetramethylsilane as internal reference normally in deuterated dimethylsulfoxide (DMSO-d_6) unless specified otherwise. Ultraviolet (UV) spectra were recorded on a Hewlett Packard HP-8450A spectrophotometer in methanol; (9 parts 0.1 N HCl/H_2O + 1 part methanol), [H^+]; and (9 parts 0.1 N $NaOH/H_2O$ + 1 part methanol), [OH^-]. Mass spectra (MS) were determined by the mass spectrometry laboratory of this department on an AEI MS-50 (with computer processing at 70 eV using a direct probe for sample introduction) for EI spectra or a KRATOS/AEI MS-9 modified for detection of either positive or negative ions for FAB spectra. (The MS-9 has a mass range of about 1400 daltons at 6 KV accelerating voltage). Infrared (IR) spectra were recorded on a Unicam SP 1000 spectrophotometer. Elemental analyses were determined by the micro-analytical laboratory of this department.

Evaporations were effected using Buchler and Büchi rotating evaporators equipped with Dewar "dry ice" condensers under water or mechanical oil pump vacuum at 40°C or cooler. Thin layer chromatography (TLC) was performed on silica gel 60-F_{254} (Merck) chromatographic sheets with sample observation under UV light (2537-Å) and/or by spraying with 5% H_2SO_4/EtOH and charring. Preparative layer chromatography (PLC) was performed on glass plates coated with Merck silica gel PF_{254}. The solvents used for TLC were 1% MeOH/$CHCl_3$, 5% MeOH/$CHCl_3$, 10% MeOH/$CHCl_3$, 20% MeOH/$CHCl_3$, 5% acetone/$CHCl_3$, isopropanol-NH_4OH-H_2O (7:1:2) and cyclohexane-acetone (3:1). Silica gel column chromatography was performed using Mallinckrodt CC-7 (200 mesh) or Merck 7734 (100-200 mesh) silica gel. Silica gel$^{\#}$ refers to the Merck 7734 (100-200 mesh) silica gel soaked in anhydrous 1,2 dimethoxyethane (presaturated with NH_3 at 0°C) at 0°C for 2 days, filtered and dried. Anion exchange chromatography was performed on Dowex 1X2 resin in the hydroxide form. Optical rotations were determined using a Perkin-Elmer 241 polarimeter at the sodium D-line at 22±2°.

All solvents and reagents of reagent grade were distilled prior to use. Purification of most solvents and reagents was accomplished according to methods described in reference (223). Dimethyl formamide was purified according to the azeotropic distillation procedure in reference (123). All dried solvents were stored over Davison 3Å and 4Å molecular sieves purchased from Fisher Scientific Company.

The reaction sequence developed for the mixed 6-N-phosphoryl nucleosides is a general three step procedure which is described in detail for the syntheses of 2',3',5'-tri-O-acetyl-6-N-(benzylphenylphosphoryl)-adenosine (80a) and 6-N-(benzylphenylphosphoryl)adenosine (81a). Subsequent experimental descriptions will refer to Procedure A: the reaction of 2',3',5'-tri-O-acetyladenosine (65) with phenyl phosphorodichloridate; Procedure B: refluxing the mixture after the addition of silver carbonate and alcohol to the solution obtained from Procedure A followed by filtration, evaporation and chromatography on silica gel#; Procedure C: deacetylation with conc. aqueous NH_3 in dioxane[131] followed by evaporation and chromatography on silica gel#.

Abbreviations used are: AIBN = azobisisobutyronitrile, DMAP = 4-(dimethylamino)pyridine, DMF = N,N-dimethylformamide, DMSO = dimethylsulfoxide, HMPA = hexamethylphosphoramide, MCPBA = m-chloroperoxybenzoic acid, PLC = preparative layer chromatography, TBDMS = t-butyldimethylsilyl, TBHP = t-butyl hydroperoxide, THF =

tetrahydrofuran, TLC = thin layer chromatography, and TPS = 2,4,6-triisopropylbenzenesulfonyl.

NMR spectral abbreviations used are: br= broad signal, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, "dd" = overlapping doublet of doublets of doublets, q = quartet, s = singlet, "s" = overlapping singlets, br s = broad singlet, t = triplet and "t" = overlapping doublet of doublets.

Mass spectral abbreviations used are: C_7H_7 = Benzyl and C_7H_7O = O-Benzyl or benzyloxy.

B) Syntheses

9-(2,3-Anhydro-β-D-lyxofuranosyl)adenine (27)

A 267 mg (1 mmol) sample of 9-(β-D-arabinofuranosyl)-adenine (32) was dissolved in 7 mL of dry DMF by heating. This solution was cooled to 0°C, 656 mg (2.5 mmol) of triphenylphosphine added and the reaction mixture stirred for 10-15 min. A solution of 400 μl (2.54 mmol) of diethyl azodicarboxylate in 7 mL of dry DMF was added over a period of 10 min at 0°C. Stirring was continued at 0°C for 15 min and then at room temperature for 2 h.

Evaporation, trituration of the residue with ether, and recrystallization from MeOH gave 192 mg (77%) of (27): mp 208-210° dec; (Lit. mp. 208-210° dec.[89]). The product had other physical data in harmony with those reported.[89,92]

9-(3-Deoxy-β-D-*threo*-pentofuranosyl)adenine (3) and 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (17)

A sample of 249 mg (1 mmol) of (27) and 1.9 g (50 mmol) of $NaBH_4$ were refluxed in 50 mL of 98% EtOH for 20 h. The solution was cooled and evaporated. The residue was dissolved in 20 mL of H_2O, the solution neutralized with 10% $AcOH/H_2O$ to pH 6, concentrated to a small volume, and applied to a column of Dowex 1X2 (OH^-) resin (2 x 20 cm). The column was eluted with H_2O, the eluate concentrated, and the residue crystallized from MeOH to give 30 mg (11%) of (17): mp 218-220°, (Lit. mp. 218.9-219.5°C[79,90], 191-192°C[87,88]); ^1H NMR (200 MHz) δ 2.3 (m, $J_{2'-2''}$ = 14.5 Hz, 1, H-2'), 2.8 (m, 1, H-2"), 3.68 (m, 2, H-5', H-5"), 3.93 (m, 1, H-4'), 4.36 (m, 1, H-3'), 4.71 ("t", $J_{OH-5',5''}$ = 5.5 Hz, 1, OH-5'), 5.99 (d, $J_{OH-3'}$ = 5.6 Hz, 1, H-3'), 6.28 (dd, $J_{1'-2'}$ = 2.5 Hz, $J_{1'-2''}$ = 8.5 Hz, 1, H-1'), 7.36 (br s, 2, NH_2), 8.17 (s, 1, H-2), 8.38 (s, 1, H-8); MS m/z 251.1018 (4.6%, $M^+[C_{10}H_{13}N_5O_3]$ = 251.1015), 221.0893 (0.6%, M^+-CH_2O), 162.0775 (56.5%, $BHCH=CH_2$), 136.0609 (36.2%, B+2H), 135.0546 (100%, B+H); Anal. ($C_{10}H_{13}N_5O_3$) Calc: C, 47.81; H, 5.22; N, 27.88. Found: C, 47.51; H, 5.15; N, 27.56.

The column then was eluted with 30% $MeOH/H_2O$, the eluate evaporated and the residue crystallized from MeOH to give 210 mg (84%) of (3): mp 198-200°C (Lit. mp. 195-196°C[79,90], 203°C[88]); ^1H NMR (200 MHz) δ 2.05 (m, 1, H-3'), 2.3 (m, 1, H-3"), 3.6 (m, 2, H-5', H-5"), 4.1 (m, 1, H-4'), 4.6 (m, 1, H-2'), 5.15 ("t", $J_{OH-5',5"}$ = 5.0 Hz, 1, OH-5'), 5.4 (d, $J_{OH-2'}$ = 5.2 Hz, 1, OH-2'), 6.15 (d, $J_{1'-2'}$ = 5.0 Hz, 1, H-1'), 7.23 (br s, 2, NH_2), 8.14 (s, 1, H-2), 8.3 (s, 1, H-8); MS m/z 251.1018 (6.2%, $M^+[C_{10}H_{13}N_5O_3]$ = 251.1019), 221.091 (8.1%, M^+-CH_2O), 164.0574 (100%, BH-CHO), 162.0778 (1.8%, $BHCH=CH_2$), 136.0616 (45.0%, B+2H), 135.0547 (97.4%, B+H); Anal. ($C_{10}H_{13}N_5O_3$) Calc: C, 47.81; H, 5.22; N, 27.88. Found: C, 47.80; H, 5.22; N, 27.87.

The above procedure was repeated several times [total 12 mmol of starting material (27)]. After neutralization with 10% $AcOH/H_2O$ the total volume was reduced to ~100 mL. Precipitated salt was removed by filtration and the filtrate subjected to continuous liquid-liquid extraction with EtOAc. Fresh EtOAc was employed periodically until most of the nucleoside was partitioned into the organic layer. The combined organic layer was cooled and filtered to remove the salt. The filtrate was concentrated to a small volume and the nucleosides were separated on a Dowex 1X2 (OH^-) column as before.

Reduction of (27) with $NaBH_4$/MeOH/t-BuOH

To a refluxing solution of 249 mg (1 mmol) of (27) and 189 mg (5 mmol) of $NaBH_4$ in 10 mL of t-BuOH was added 1.6 mL of MeOH dropwise for about 1 h and the resulting solution refluxed for an additional 2 h. The solution was cooled, concentrated, the residue dissolved in a minimum amount of H_2O and adjusted to pH 6 with 10% AcOH/H_2O. The products (3) and (17) were obtained by chromatographic separation on the Dowex 1X2 (OH^-) resin as described previously. Recrystallization from MeOH afforded 27 mg (11%) of (17) and 130 mg (52%) of (3). The physical properties of both (17) and (3) were identical with the previous data.

2'-O-(2,4,6-Triisopropylbenzenesulfonyl)adenosine and 3'-O-(2,4,6-triisopropylbenzenesulfonyl)adenosine (36)

A 534 mg (2 mmol) sample of adenosine (35) was dissolved in 14 mL of dry DMF by heating. This solution was cooled to -23°C in CCl_4/dry ice and treated with 130 mg (5.4 mmol) of sodium hydride (50% dispersion in oil was washed with dry benzene under an atmosphere of nitrogen and transferred to the above solution in ~3 mL of dry DMF at -23°C). This mixture was stirred at -23°C for ~20 min, treated with 726 mg (2.4 mmol) of TPS-Cl, and then stirred at -23°C for 6 h. The solution was evaporated to a pale yellow oil which was chromatographed on silica gel with 5% MeOH/$CHCl_3$ as eluant to yield 853 mg (80%) of a mixture of the title compounds with: MS m/z 533.2289 (1.4%, $M^+[C_{25}H_{35}N_5O_6S]$ = 533.2308), 162.0776 (1.6%, BHCH=CH_2), 136.0619 (100%, B+2H), 135.0544 (79.6% B+H).

9-(3-Deoxy-β-D-*threo*-pentofuranosyl)adenine (3) and 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (17)

A solution of 853 mg (1.6 mmol) of the above mixture (36) in 41 mL of dry DMSO was treated with 20.5 mL of 1 *M* $LiEt_3BH$/THF and the resulting solution stirred in an ice-bath for 2 h, and then at room temperature for 40 h. The reaction mixture was carefully quenched with 50 mL of H_2O and purged with nitrogen gas. Concentration of the solution *in vacuo* and chromatography of the syrup on a column of Dowex 1X2 (OH^-) resin using H_2O, followed by 30% MeOH/H_2O as eluants afforded 132 mg (17%) of (17): mp 218-220°C and 278 mg (35%) of (3): mp 198-200°C respectively, after recrystallization from MeOH. Both compounds had identical physical properties with the previous compound prepared in the above sequence [(27)→(17)+(3)].

2',5'-Di-O-(t-butyldimethylsilyl)-3'O-phenoxythiocarbonylarabinosyladenine and 3',5'-di-O-(t-butyldimethylsilyl)-2'-O-phenoxythiocarbonylarabinosyladenine (39)

Arabinosyladenine (32) was silylated using TBDMS-Cl to yield a mixture of 2',5'-di-O-(t-butyldimethylsilyl)-arabinosyladenine and 3',5'-di-O-(t-butyldimethylsilyl)-arabinosyladenine (38) in a 10:3 ratio.[103]

A sample of 496 mg (1 mmol) of the above mixture (38) was suspended in 15 mL of dry CH_3CN. 366 mg (3 mmol) of DMAP and 200 μl (1.45 mmol) of phenoxythiocarbonyl chloride were added and the resulting solution stirred at room temperature for 15 h. The volatile solvents were evaporated and the residue partitioned between EtOAc and H_2O. The organic phase was washed successively with sat. aq. $NaHCO_3$, H_2O, sat. aq. NaCl and then dried over Na_2SO_4 and evaporated to give a foam which was chromatographed on silica gel using 3% $MeOH/CHCl_3$ as eluant to afford 475 mg (75%) of the title compounds as a mixture.

9-(3-Deoxy-β-D-*threo*-pentofuranosyl)adenine (3) and 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)adenine (41)

A solution of 475 mg (0.75 mmol) of the above mixture (39) in 7.8 mL of dry toluene was treated with 895 μl (3.32 mmol) of *n*-Bu_3SnH and 95 mg (0.58 mmol) AIBN and the reaction mixture stirred overnight at 75°C. Evaporation of volatile solvents afforded a residue which was treated with 3 mL of 1 *M* *n*-Bu_4NF/THF. Solvents were evaporated *in vacuo* and the residue was partitioned between ether and H_2O. The aqueous phase was applied to a column of Dowex 1X2 (OH^-) resin. The column was eluted with 15% MeOH/H_2O, the eluate concentrated and the residue recrystallized from Et_2O/EtOH to give 17.5 mg (9%) of (41): mp 191-192°C (Lit. mp. 191-192°C[105]). The product had other physical data in harmony with those reported.[105] The column was then eluted with 30% MeOH/H_2O, the eluate evaporated and the residue recrystallized from MeOH to afford 88 mg (45%) of (3): mp 198-200°C. This compound had identical physical properties with the previous data.

3,5,6-Tri-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (44)

To a solution of 2.2 g (10 mmol) of 1,2-*O*-isopropylidene-α-D-glucofuranose (43) in 25 mL DMSO was added 3.92 g (70 mmol) of finely powdered KOH and the reaction mixture stirred for ~15 min. Benzyl bromide (4.28 mL, 36 mmol) was added dropwise with stirring to the above solution in a cold water bath. The reaction mixture was allowed to stir at room temperature for ~14 h, at which time TLC (silica gel, 5% acetone/$CHCl_3$) indicated complete reaction. The excess of benzyl bromide was decomposed by the addition of MeOH (water cooling) and stirring for 30 min. The reaction mixture was evaporated to dryness and the residue taken up in 100 mL of CH_2Cl_2. The organic layer was washed with water (4 x 25 mL), dried over Na_2SO_4 and evaporated. The residue was distilled at 260-270°C/0.1 Torr (Lit. bp. 260-280°C/0.1 Torr[108]) to give 4.3 g (88%) of (44): $[\alpha]_D^{20} = -35°$ (c = 0.227, $CHCl_3$); ^1H NMR (200 MHz) δ 1.3, 1.4 (2s, 2 x 3, 2 x CH_3), 3.6 (dd, J_{6-5} = 6.0 Hz, $J_{6-6'}$ = 11.0 Hz, 1, H-6), 3.85 (dd, $J_{6'-5}$ = 2.0 Hz, $J_{6'-6}$ = 11.0 Hz, 1, H-6'), 3.92 (ddd, $J_{5-6'}$ = 2.0 Hz, J_{5-6} = 6.0 Hz, J_{5-4} = 9.0 Hz, 1, H-5), 4.01 (d, J_{3-4} = 3.0 Hz, 1, H-3), 4.19 (dd, J_{4-3} = 3.0 Hz, J_{4-5} = 9.0 Hz, 1, H-4), 4.46-4.7 (m, 6, 3 x CH_2Ph), 4.8

(d, J_{2-1} = 4.4 Hz, 1, H-2), 5.83 (d, J_{1-2} = 4.4 Hz, 1, H-1), 7.2-7.4 (m, 15, 3 x C_6H_5); MS m/z 399.1807 (8.3%, M^+-C_7H_7[$C_{23}H_{27}O_6$] = 399.1806), 308.1215 (0.1%, M^+-2xC_7H_7), 91.0549 (100%, C_7H_7); Anal. ($C_{30}H_{34}O_6$) Calc: C, 73.45; H, 6.99. Found: C, 73.40; H, 7.00.

2,3,5,6-Tetra-*O*-benzyl-D-glucofuranose (47)

A sample of 3.74 g (6.75 mmol) of met[illegible]l 2,3,5,6-tetra-*O*-benzyl-D-glucofuranoside (46) was dissolved in 25 mL of DMSO. To this solution, 38 mL of 60% AcOH and 380 mg of $CaBr_2$ were added and the reaction mixture refluxed overnight. The volatiles were evaporated and the residue co-evaporated w[illegible] toluene. The resulting yellow colored oil was extracted into 50 mL of Et_2O. The organic layer was successively washed with sat. $NaHCO_3$, H_2O, sat. NaCl, dried over Na_2SO_4 and evaporated to give 3.31 g (91%) of (47): ^{1}H NMR (400 MHz) δ 5.13 (dd, 1, H-1β), 5.35 (dd, 1, H-1α), 6.25 (d, J_{OH-1} = 6.0 Hz, 1, OH-α), 6.5 (d, J_{OH-1} = 6.0 Hz, 1, OH-β), 7.2-7.4 (m, C_6H_5's); MS m/z 431.1855 (0.9%, M^+-C_7H_7-H_2O[$C_{27}H_{27}O_5$] = 431.1858), 91.055 (100%, C_7H_7); Anal. ($C_{34}H_{36}O_6$) Calc: C, 75.53; H, 6.71. Found: C, 75.85; H, 6.90.

Methyl 2,3,5,6-tetra-O-benzyl-α-D-glucofuranoside (50)

A 300 mg (0.645 mmol) sample of methyl 3,5,6-tri-O-benzyl-α-D-glucofuranoside (48) was dissolved in 1.5 mL of DMSO. To this solution was added 144 mg (2.57 mmol) of finely powdered KOH and the reaction mixture stirred at room temperature for ~15 min. Benzyl bromide (92 μl, 0.77 mmol) was added dropwise with stirring to the above solution and stirring continued for 15 min. The reaction was worked up as described previously for the preparation of (44). The pure product was obtained by PLC using 5% acetone/$CHCl_3$ as the developing solvent to yield 330 mg (92%) of (50): $[\alpha]_D^{20} = +36°$ (c = 0.218, $CHCl_3$); ^{1}H NMR (400 MHz) δ 3.34 (s, 3, CH_3), 3.6 (dd, $J_{6'-5}$ = 6.0 Hz, $J_{6'-6}$ = 10.0 Hz, 1, H-6'), 3.8 (dd, $J_{6-6'}$ = 10.0 Hz, J_{6-5} = 1.8 Hz, 1, H-6), 3.9 (ddd, J_{5-4} = 7.0 Hz, $J_{5-6'}$ = 6.0 Hz, J_{5-6} = 1.8 Hz, 1, H-5), 4.05 ("t", J_{2-3} = 4.0 Hz, J_{2-1} = 4.0 Hz, 1, H-2), 4.12 (dd, J_{3-4} = 6.0 Hz, J_{3-2} = 4.0 Hz, 1, H-3), 4.22 ("t", J_{4-3} = 6.0 Hz, J_{4-5} = 7.0 Hz, 1, H-4), 4.46-4.7 (m, 8, 4 x CH$_2$Ph), 5.02 (d, J_{1-2} = 4.0 Hz, 1, H-1), 7.2-7.4 (m, 20, 4 x C_6H_5); MS m/z 431.1850 (0.7%, $M^+-C_7H_7-CH_3OH[C_{27}H_{27}O_5]$ = 431.1858), 91.0548 (100%, C_7H_7); Anal. ($C_{35}H_{38}O_6$) Calc: C, 75.79; H, 6.91. Found: C, 75.49; H, 6.86.

Methyl 2,3,5,6-tetra-O-benzyl-β-D-glucofuranoside (51)

The title compound was obtained as described in the preceeding procedure for (50) using methyl 3,5,6-tri-O-benzyl-β-D-glucofuranoside (49) as the starting material. The pure product was obtained by PLC using 5% acetone/ $CHCl_3$ as the developing solvent to afford 340 mg (95%) of (51): $[\alpha]_D^{20} = -30°$ (c = 0.105, $CHCl_3$); ^1H NMR (400 MHz) δ 3.26 (s, 3, CH_3), 3.62 (dd, $J_{6'-5}$ = 6.0 Hz, $J_{6'-6}$ = 11.0 Hz, 1, H-6'), 3.87 (dd, $J_{6-6'}$ = 11.0 Hz, J_{6-5} = 1.8 Hz, 1,H-6), 3.9 (ddd, J_{5-4} = 8.5 Hz, J_{5-6} = 1.8 Hz, $J_{5-6'}$ = 6 Hz, 1, H-5), 4.01 (s, 1, H-2), 4.06 (d, J_{3-4} = 4.5 Hz, 1 H-3), 4.2 (dd, J_{4-3} = 4.5 Hz, J_{4-5} = 8.5 Hz, 1, H-4), 4.42-4.68 (m, 8, 4 x CH_2Ph), 4.9 (s, 1, H-1), 7.2-7.4 (m, 20, 4 x C_6H_5); MS m/z 431.1848 (2.2%, $M^+-C_7H_7-CH_3OH$ [$C_{27}H_{27}O_5$] = 431.1858), 91.0548 (100%, C_7H_7); Anal. ($C_{35}H_{38}O_6$) Calc. C, 75.79; H, 6.91. Found: C, 75.39; H, 6.82.

Ethyl (E)4-methylpent-2-enoate (56)

A solution of 3.6 g (50 mmol) of isobutyraldehyde (55), 7.92 g (66 mmol) of ethyl hydrogen malonate (54), 9 mL of pyridine and 490 μl (0.5 mmol) of piperidine was refluxed until the evolution of CO_2 was complete. The resulting solution was cooled, poured on ice and neutralized with 2 N HCl. Ether (100 mL) was added and the organic layer washed successively with dil. HCl, sat. $NaHCO_3/H_2O$, H_2O, sat. $NaCl/H_2O$, dried over Na_2SO_4 and evaporated to give a yellow colored oil. Distillation of this oil at 60-62°C/0.1 Torr (Lit. bp. 62.4°C/0.11 Torr[124-126]) afforded 4.8 g (67%) of (56) as a clear oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 1.05 ("d", 6, $-CH\underline{Me_2}$), 1.3 (t, J = 7.0 Hz, 3, $-CH_2\underline{CH_3}$), 2.45 ("ds"*, J_{4-CMe_2} = 7.0 Hz, J_{4-2} = 1.5 Hz, 1, H-4 [$-C\underline{H}Me_2$]), 4.2 (q, J = 7.0 Hz, 2, $\underline{-CH_2}CH_3$), 5.8 (dd, J_{2-4} = 1.5 Hz, J_{2-3} = 16.0 Hz, H-2), 7.0 (dd, J_{3-2} = 16.0 Hz, J_{3-4} = 7.0 Hz, 1, H-3); MS m/z 142.0994 (15.3%, $M^+[C_8H_{14}O_2]$ = 142.0994), 113.0603 (37.1%, $M^+-C_2H_5$), 97.0651 (10.9%, $M^+-C_2H_5O$).

Ethyl DL-threo-2,3-dihydroxy-4-methylpentanoate (57)

A 7.2 g (50 mmol) sample of (56), 3.2 g of Et_4NOAc, 100 mL of reagent acetone and 12 mL of 70% TBHP were combined in a 500 mL round bottom flask and stirred at room temperature for 15 min. This solution was cooled in ice and 8 mL of stock solution** of OsO_4/t-BuOH was

* "ds" = doublet of septets.

** Stock solution of OsO_4/t-BuOH: 50 mg of OsO_4 was dissolved in 10 mL of t-BuOH and 600 μl of 70% TBHP added to this solution. Each mL contains ~5 mg of OsO_4.

added. The resulting golden yellow solution was stirred in an ice bath for ~1 h and then at room temperature overnight. Diethyl ether (200 mL) and 30 mL of freshly prepared 10% $NaHSO_3/H_2O$ were added to the above solution which was cooled in ice and stirred for ~1 h. Solid NaCl was added and the solution stirred at room temperature for 2 h and transferred into a separatory funnel. The aqueous layer was washed twice with ether. The combined organic layer was washed with sat. $NaCl/H_2O$, dried over Na_2SO_4 and concentrated to afford a yellow colored oil. Distillation of this oil at 85°C/0.4 Torr gave 7.1 g (80%) of (57) as a clear colorless oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 1.02 ("d", J = 7.5 Hz, 6, $-\underline{CHMe_2}$), 1.35 (t, J = 7.0 Hz, 3, $-CH_2\underline{CH_3}$), 1.9 (m, 1, H-4 [$-C\underline{H}Me_2$]), 3.51 (dd, J_{2-3} = 2.0 Hz, 1, H-3), 4.28 (m, 3, $-\underline{CH_2}CH_3$, H-2), 3.1 (br, 2, OH-2, OH-3); MS m/z 177.1129 (3.4%, $MH^+[C_8H_{17}O_4]$ = 177.1127), 158.0954 (0.8%, M^+-H_2O); Anal. ($C_8H_{16}O_4$) Calc: C, 54.53; H, 9.15. Found: C, 54.40; H, 9.17.

DL-threo-2,3-dihydroxy-4-methylpentanamide (7')

A sample of 1.76 g (10 mmol) of (57) was dissolved in 5 mL of NH_3/MeOH and this solution stirred at room temperature for ~3 h. Evaporation of volatiles gave a solid which was recrystallized from MeOH to yield 1.05 g

(72%) of (7'): mp 193-194°C (Lit. mp. 192-194°C[64]); ^1H NMR ($CDCl_3$, 100 MHz) δ 0.8, 0.92 (2 d, J = 7.0 Hz, 2 x 3, -CHMe$_2$), 1.6 (m, 1, H-4[-CHMe$_2$]), 3.3 (dd, J_{3-2} = 2.0 Hz, J_{3-OH-3} = 8.0 Hz, 1, H-3), 3.8 (dd, J_{2-3} = 2.0 Hz, J_{2-OH-2} = 7.0 Hz, 1, H-2), 4.3 (d, 1, OH-3), 4.97 (d, 1, OH-2), 7.15 (br s, 2, NH_2); MS m/z 129.0791 (1.8%, $M^+-H_2O[C_6H_{11}NO_2]$ = 129.0790); Anal. ($C_6H_{13}NO_3$) Calc: C, 48.97; H, 8.90; N, 9.52. Found: C, 48.88; H, 8.81; N, 9.49.

2',3',5'-Tri-*O*-acetyl-6-*N*-(diphenylphosphoryl)-adenosine (58)

To a refluxing solution of 129 μl (0.86 mmol) of phenyl phosphorodichloridate in 3 mL of dry benzene was added 236 mg (0.6 mmol) of 2',3',5'-tri-*O*-acetyladenosine (65) followed by 6 mL more of dry benzene. The reaction mixture was refluxed for ~10 h until it became clear. At this stage, 113 mg (1.2 mmol) of phenol was added and the reaction mixture refluxed for 4 h. Evaporation of volatiles *in vacuo* and chromatography of the residue on silica gel# using 2% MeOH/$CHCl_3$ as eluant afforded 240 mg (64%) of (58): mp 69-71°C (Lit. mp. 78-80°C[131]); UV (MeOH) max 260 nm (ε 22,000), min 226 nm (ε 5,000); (H^+) max 260 nm (ε 21,000), min 230 nm (ε 4,600); (OH^-) max 277

nm (ε 33,000), min 238 nm (ε 5,500); ^{1}H NMR (200 MHz) δ 2.00, 2.05, 2.13 (3 s, 3 x 3, 3 x OAc), 4.26 (m, $J_{5'-5''}$ = 12.5 Hz, $J_{5'-4'}$ = 6.5 Hz, 1, H-5'), 4.4 (m, 2, H-4', H-5"), 5.64 ("t", $J_{3'-4'}$ = 5.0 Hz, $J_{3'-2'}$ = 5.5 Hz, 1, H-3'), 6.02 ("t", $J_{2'-3'}$ = 5.5 Hz, $J_{2'-1'}$ = 5.5 Hz, 1 H-2'), 6.3 (d, $J_{1'-2'}$ = 5.5 Hz, 1, H-1'), 7.15-7.5 (m, 10, 2 x C_6H_5), 8.62 ("s", 2, H-2, H-8), 10.5 (br, 1, NH); MS m/z (glycerol/FAB) = 626 (MH^+); Anal. ($C_{28}H_{28}N_5O_{10}P$) Calc: C, 53.76; H, 4.51; N, 11.19. Found: C, 53.75; H, 4.50; N, 10.90.

2',3',5'-Tri-O-acetyl-6-N-[(2-cyanoethyl)phenylphosphoryl]adenosine (59)

Procedure A (0.1 mmol) was followed by Procedure B using 40 mg of Ag_2CO_3 and 13.7 μl (0.2 mmol) of 2-cyanoethanol, reflux time being 2 h. Purification of the residue on silica gel# afforded 35 mg (58%) of (59): mp 62-64°C (Lit. mp. 62-65°C[131]). The physical properties of (59) were in harmony with the published data.[131]

2',3',5'-Tri-O-acetyl-6-N-[(benzyl)phenylphosphoryl]-adenosine (80a)

To a refluxing solution of 215 μl (1.44 mmol) of phenyl phosphorodichloridate in 10 mL of dry benzene was added 393 mg (1 mmol) of 2',3',5'-tri-O-acetyladenosine (65). The reaction mixture was refluxed for ~10 h until it became clear and no starting material was observed on TLC (silica gel, 5% MeOH/$CHCl_3$). It was then cooled to room temperature (Procedure A). At this stage 400 mg (1.45 mmol) of Ag_2CO_3 and 207 μl (2 mmol) of benzyl alcohol were added and the reaction mixture refluxed for an additional 4 h. It was then cooled, filtered, evaporated and the residue chromatographed on silica gel# with 2% MeOH/$CHCl_3$ as the eluant (Procedure B). Evaporation of appropriate fractions gave 300 mg (63%) of (80a) as an amorphous solid: mp 79-81°C; UV (MeOH) max 259 nm (ε 20,200), min 234 nm (ε 13,800); (H^+) max 260 nm (ε 18,600), min 229 nm (ε 9,800); (OH^-) max 276 nm (ε 29,000), min 241 nm (ε 10,000); ^1H NMR (200 MHz) δ 2.00, 2.04, 2.13 (3 s, 3 x 3, 3 x OAc), 4.28 (m, $J_{5'-4'}$ = 6.5 Hz, $J_{5'-5''}$ = 12.5 Hz, 1, H-5'), 4.42 (m, 2, H-4', H-5"), 5.3 (d, $J_{H-C-O-P}$ = 7.5 Hz, 2, $-CH_2Ph$), 5.65 ("t", $J_{3'-4'}$ = 5.0 Hz, $J_{3'-2'}$ = 5.5 Hz, 1, H-3'), 6.04 ("t", $J_{2'-1'}$ = 5.5 Hz, $J_{2'-3'}$ = 5.5 Hz, 1, H-2'), 6.3 (d, $J_{1'-2'}$ = 5.5 Hz, 1, H-1'), 7.18-7.5 (m, 10, 2 x C_6H_5), 8.58 (s, 1, H-2), 8.62 (s, 1, H-8), 10.28 (br, 1, NH); MS m/z (glycerol/FAB) = 640 (MH^+); Anal. ($C_{29}H_{30}N_5O_{10}P$) Calc: C, 54.46; H, 4.73; N, 10.95. Found: C, 54.76; H, 4.81; N, 10.65.

2',3',5'-Tri-O-acetyl-6-N-[(ethyl)phenylphosphoryl]-adenosine (80b)

Procedure A (1 mmol) was followed by Procedure B using 400 mg of Ag_2CO_3 and 117 µl (2 mmol) of ethyl alcohol. The reaction mixture was refluxed for 2 h. Purification of the residue on silica gel[#] gave 346 mg (60%) of (80b): mp 57-59°C; UV (MeOH) max 258 nm (ε 18,100), min 225 nm (ε 4,600); (H^+) max 260 nm (ε 16,200), min 225 (ε 4,900); (OH^-) max 276 nm (ε 23,000), min 239 nm (ε 2,800); ^1H NMR (200 MHz) δ 1.28 (t, J = 7.5 Hz, 3, $-CH_2\underline{CH_3}$), 2.01, 2.05, 2.14 (3 s, 3 x 3, 3 x OAc), 4.26 (m, 2, $-\underline{CH_2}CH_3$), 4.35 (m, 3, H-4', H-5', H-5"), 5.65 ("t", $J_{3'-4'}$ = 5.0 Hz, $J_{3'-2'}$ = 5.5 Hz, 1, H-3'), 6.05 ("t", $J_{2'-3'}$ = 5.5 Hz, $J_{2'-1''}$ = 5.5 Hz, 1, H-2'), 6.3 (d, $J_{1'-2'}$ = 5.5 Hz, 1, H-1'), 7.16-7.45 (m, 5, C_6H_5), 8.58 (s, 1, H-2), 8.63 (s, 1, H-8), 10.15 (br, 1, NH); MS m/z (glycerol-sulfolane/FAB) = 578 (MH^+); Anal. ($C_{24}H_{28}N_5O_{10}P$) Calc: C, 49.92; H, 4.89; N, 12.13. Found: C, 49.69; H, 4.93; N, 11.97.

2',3',5'-Tri-O-acetyl-6-N-([2-(2-ethoxyethoxy)ethyl]phenylphosphoryl)adenosine (80c)

Procedure A (1 mmol) was followed by Procedure B using 400 mg of Ag_2CO_3 and 300 μl (2.2 mmol) of 2-(2-ethoxyethoxy)ethanol, reflux time was 3 h. Purification of the residue on silica gel# afforded 513 mg (77%) of (80c): mp 38-40°C; UV (MeOH) max 259 nm (ϵ 19,100), min 226 nm (ϵ 10,300); (H^+) max 260 nm (ϵ 19,000), min 227 nm (ϵ 6,500); (OH^-) max 276 nm (ϵ 27,800), min 240 nm (ϵ 4,500); ^1H NMR (200 MHz) δ 1.05 (t, 3, $-CH_2CH_3$), 2.0, 2.04, 2.12 (3 s, 3 x 3, 3 x OAc), 3.44 (m, 8, 4 x CH_2), 4.3 (m, 5, H-4', H-5', H-5", $-P(O)OCH_2$), 5.64 ("t", $J_{3'-4'}$ = 5.0 Hz, $J_{3'-2'}$ = 5.8 Hz, 1, H-3'), 6.03 ("t", $J_{2'-3'}$ = 5.8 Hz, $J_{2'-1'}$ = 5.0 Hz, 1, H-2'), 6.29 (d, $J_{1'-2'}$ = 5.0 Hz, 1, H-1'), 7.15-7.5 (m, 5, C_6H_5), 8.56 (s, 1, H-2), 8.62 (s, 1, H-8), 10.1 (br, 1, NH); MS m/z (glycerol-sulfolane/FAB) = 666 (MH^+); Anal. ($C_{28}H_{36}N_5O_{12}P$) Calc: C, 50.53; H, 5.45; N, 10.52. Found: C, 50.27; H, 5.74; N, 10.21.

2',3',5'-Tri-O-acetyl-6-N-[(isopropyl)phenylphosphoryl]adenosine (80d)

Procedure A (1 mmol) was followed by Procedure B using 400 mg of Ag_2CO_3 and 153 μl (2 mmol) of isopropyl alcohol, reflux time was 2 h. Purification of the residue on silica gel[#] afforded 302 mg (65%) of (80d): mp 35-37°C; UV (MeOH) max 259 nm (ε 19,800), min 225 nm (ε 4,100); (H^+) max 260 nm (ε 20,200), min 225 nm (ε 7,000); (OH^-) max 276 nm (ε 27,700) min 239 nm (ε 1,100); ^{1}H NMR (200 MHz) δ 1.3 (d, J = 6.5 Hz, 6, -CH$\underline{\text{Me}_2}$), 2.0, 2.05, 2.21 (3 s, 3 x 3, 3 x OAc), 4.28 and 4.85 (m, 1, -C$\underline{\text{H}}$Me$_2$), 4.3 (m, 3, H-4', H-5', H-5"), 5.66 ("t", $J_{3'-2'}$ = 5.0 Hz, $J_{3'-4'}$ = 5.0 Hz, 1, H-3'), 6.04 ("t", $J_{2'-3'}$ = 5.0 Hz, $J_{2'-1'}$ = 5.5 Hz, 1, H-2'), 6.3 (d, $J_{1'-2'}$ = 5.5 Hz, 1, H-1'), 7.1-7.6 (m, 5, C_6H_5), 8.58 (s, 1, H-2), 8.62 (s, 1, H-8), 10.08 (br, 1, NH); MS m/z (glycerol/FAB) = 592 (MH^+).

6-*N*-[(Benzyl)phenylphosphoryl]adenosine (81a)

A sample of 33 mg (0.05 mmol) of (80a) dissolved in 80 μl of dioxane was stirred overnight with 360 μl of conc. aqueous ammonia at room temperature. The reaction mixture was evaporated to dryness and the residue purified by chromatography on silica gel[#] column (2 x 10 cm) using 5% MeOH/$CHCl_3$ as the eluant (Procedure C). Recrystallization from $CHCl_3$ by diffusion of Et_2O afforded 22 mg (83%) of (81a): mp 79-81°C; UV (MeOH) max 261 nm (ε 20,600),

min 233 nm (ε 11,800); (H^+) max 261 nm (ε 19,700), min 230 nm (ε 7,500); (OH^-) max 276 nm (ε 28,200), min 240 nm (ε 6,900); ^1H NMR (200 MHz) δ 3.77 (m, $J_{5'-5''}$ = 12.5 Hz, 2, H-5', H-5"), 4.0 (m, 1, H-4'), 4.2 (m, 1, H-3'), 4.63 ("dd", $J_{2'-1'}$ = 6.0 Hz, $J_{2'-3'}$ = 4.0 Hz, $J_{2'-OH-2}$ = 6.0 Hz, 1, H-2'), 5.2 (m, 2, OH-3', OH-5'), 5.24 (d, $J_{H-C-O-P}$ = 8.0 Hz, 2, $-\underline{CH_2}Ph$), 5.55 (d, $J_{OH-2'}$ = 6.0 Hz, 1, OH-2'), 6.0 (d, $J_{1'-2'}$ = 6.0 Hz, 1, H-1'), 7.15-7.5 (m, 10, 2 x C_6H_5), 8.56 (s, 1, H-2), 8.66 (s, 1, H-8), 10.3 (br, 1, NH); MS m/z (glycerol/FAB) = 514 (MH^+); Anal. ($C_{29}H_{30}N_5O_{10}P \cdot 1.25\ H_2O$) Calc: C, 51.54; H, 4.98; N, 13.07. Found: C, 51.59; H, 4.75; N, 13.06.

6-*N*-[(Ethyl)phenylphosphoryl]adenosine (81b)

Procedure C was applied to 90 mg (0.16 mmol) of (80b) using 180 μl of dioxane and 800 μl of conc. aq. NH_3 to give 65 mg (93%) of (81b) after recrystallization from $CHCl_3$ by diffusion of Et_2O: mp 87-89°C; UV (MeOH) max 260 nm (ε 18,700); min 227 nm (ε 3,200); (H^+) 261 nm (ε 16,500), min 228 nm (ε 4,100); (OH^-) 275 nm (ε 22,000), min 239 nm (ε 2,400); ^1H NMR (200 MHz) δ 1.28 (t, J = 8.0 Hz, 3, $-CH_2\underline{CH_3}$), 3.65 (m, 2, H-5', H-5"), 3.95 (m, 1, H-4'), 4.25 (m, 3, H-3', $-\underline{CH_2}CH_3$), 4.61 ("dd", $J_{2'-3'}$ = 4.5 Hz, $J_{2'-1''}$ = 5.8 Hz, $J_{2'-OH-2}$ = 5.8 Hz, 1, H-2'), 5.24

(m, 2, OH-3', OH-5'), 5.54 (d, 1, OH-2'), 6.0 (d, $J_{1'-2'}$ = 5.8 Hz, 1, H-1'), 7.15-7.5 (m, 5, C_6H_5), 8.53 (s, 1, H-2), 8.64 (s, 1, H-8), 10.08 (br, 1, NH); MS m/z (glycerol/FAB) = 452 (MH^+); Anal. ($C_{18}H_{22}N_5O_7P\cdot0.25\ H_2O$) Calc: C, 47.42; H, 4.97; N, 15.36. Found: C, 47.48; H, 4.94; N, 15.56.

6-N-{[2-(2-Ethoxyethoxy)ethyl]phenylphosphoryl}-adenosine (81c)

Procedure C was applied to 150 mg (0.225 mmol) of (80c) using 300 µl of dioxane and 1.35 mL of conc. aq. NH_3 to give 104 mg (86%) of (81c) after recrystallization from $CHCl_3$ by diffusion of Et_2O: mp 39-41 °C; UV (MeOH) max 260 nm (ε 19,600), min 234 nm (ε 11,400); (H^+) max 260 nm (ε 18,700), min 228 nm (ε 7,600); (OH^-) max 276 nm (ε 26,200), min 239 nm (ε 4,900); ^1H NMR (200 MHz) δ 1.04 (t, J = 8.0 Hz, 3, $-CH_2\underline{CH_3}$), 3.52 (m, 10, 4 x CH_2, H-5', H-5"), 3.98 (m, 1, H-4'), 4.18 (ddd, $J_{3'-4'}$ = 4.0 Hz, $J_{3'-2'}$ = 5.0 Hz, $J_{3'-OH-3'}$ = 4.8 Hz, 1, H-3'), 4.3 (m, 2, $-P(O)-OCH_2-$), 4.6 ("dd", $J_{2'-1'}$ = 5.8 Hz, $J_{2-3'}$ = 5.0 Hz, $J_{2'-OH-2'}$ = 6.0 Hz, 1, H-2'), 5.15 (t, $J_{OH-5',5''}$ = 5.5 Hz, 1, OH-5'), 5.23 (d, $J_{OH-3'}$ = 4.8 Hz, 1, OH-3'), 5.5 (d, $J_{OH-2'}$ = 6.0 Hz, 1, OH-2'), 5.8 (d, $J_{1'-2'}$ = 5.8 Hz, 1, H-1'), 7.15-7.5 (m, 5, C_6H_5), 8.53 (s, 1, H-2), 8.64 (s, 1, H-8), 10.04 (br, 1, NH); MS m/z (glycerol/FAB) = 540

(MH$^+$); Anal. ($C_{22}H_{30}N_5O_9P \cdot 0.25\ H_2O$) Calc: C, 48.58; H, 5.65; N, 12.87. Found: C, 48.23; H, 5.89; N, 13.19.

6-N-[(Isopropyl)phenylphosphoryl]adenosine (81d)

Procedure C was applied to 100 mg (0.17 mmol) of (80d) using 190 μl of dioxane and 820 μl of conc. aq. NH_3 to yield 70 mg (87%) of (81d) after recrystallization from $CHCl_3$ by diffusion of Et_2O: mp 93-95°C; UV (MeOH) max 260 nm (ϵ 19,200), min 225 nm (ϵ 2,600); (H$^+$) max 261 nm (ϵ 19,800), min 226 nm (ϵ 4,400); (OH$^-$) max 276 nm (ϵ 28,000), min 239 nm (ϵ 3,600); ^{1}H NMR (200 MHz) δ 1.3 (d, J = 6.5 Hz, 6, -CHMe$_2$), 3.65 (m, 2, H-5', H-5"), 4.0 (m, 1, H-4') 4.19 (m, 1, H-3'), 4.28 and 4.88 (m, 1, -CHMe$_2$), 4.63 (m, 1, H-2'), 5.18 (t, $J_{OH-5',5''}$ = 5.2 Hz, 1, OH-5'), 5.25 (d, $J_{OH-3'}$ = 4.5 Hz, 1, OH-3'), 5.52 (d, $J_{OH-2''}$ = 6 Hz, 1, OH-2'), 5.98 (d, $J_{1'-2'}$ = 5.5 Hz, 1, H-1'), 7.15-7.55 (m, 5, C_6H_5), 8.55 (s, 1, H-2), 8.64 (m, 1, H-8), 10.08 (br, 1, NH); MS m/z (glycerol/FAB) = 466 (MH$^+$); Anal. ($C_{19}H_{24}N_5O_7P \cdot 0.25\ H_2O$) Calc: C, 48.56; H, 5.26; N, 14.90. Found: C, 48.48; H, 5.06; N, 15.20.

2',3',5'-Tri-O-acetyl-6-N-(phenylphosphoryl)adenosine (86)

A 30 mg (0.047 mmol) sample of (80a) was hydrogenolyzed with 10 mg of 5% Pd-C catalyst in 25 mL of 95% EtOH at 30 psi H_2 for 10 h at room temperature in a Parr shaking apparatus. Filtration of the mixture with a celite pad, evaporation of the solvent and recrystallization of the residue from $CHCl_3$ by diffusion of Et_2O gave 23 mg (89%) of (86): mp 89-91°C; UV (MeOH) max 263 nm (ε 15,600), min 229 nm (ε 2,000); (H^+) max 267 nm (ε 16,700), min 232 nm (ε 3,500); (OH^-) max 263 nm (ε 16,900), min 236 nm (ε 5,900); ^1H NMR (200 MHz) δ 2.0, 2.04, 2.11 (3 s, 3 x 3, 3 x OAc) 4.32 (m, 3, H-4', H-5', H-5"), 5.6 ("t", $J_{3'-2'}$ = 5.8 Hz, $J_{3'-4'}$ = 5.5 Hz, 1, H-3'), 5.7 (br, 1, OH), 5.92 ("t", $J_{2'-1'}$ = 5.0 Hz, $J_{2'-3'}$ = 5.8 Hz, 1, H-2'), 6.28 (d, $J_{1'-2'}$ = 5.0 Hz, 1, H-1'), 7.0-7.3 (m, 5, C_6H_5), 8.64 (s, 1, H-2), 8.67 (s, 1, H-8), 10.08 (br, 1, NH); MS m/z (glycerol/FAB) = 550 (MH^+); Anal. ($C_{22}H_{24}N_5O_{10}P\cdot 0.5\ H_2O$) Calc: C, 47.70; H, 4.45; N, 12.64. Found: C, 47.50; H, 4.46; N, 12.52.

6-N-(Phenylphosphoryl)adenosine (61)

A 30 mg (0.058 mmol) sample of (81a) was hydrogenolyzed with 10 mg of 10% Pd-C catalyst in 25 mL of 95% EtOH at 30 psi H_2 for ~10 h at room temperature in a Parr shaking apparatus. Filtration of the mixture with a celite pad, evaporation of the solvent and recrystallization of the resulting residue from EtOH by diffusion of Et_2O afforded 18 mg (73%) of (61): mp 159-161°C; UV (MeOH) max 263 nm (ϵ 16,000), min 235 nm (ϵ 4,200); (H^+) max 268 nm (ϵ 16,100), min 235 nm (ϵ 5,000); (OH^-) max 263 nm (ϵ 16,200), min 235 nm (ϵ 5,400); ^1H NMR (200 MHz) δ 3.63 (m, 2, H-5', H-5"), 3.98 (m, 1, H-4'), 4.16 ("t", $J_{3'-2'}$ = 5.0 Hz, $J_{3'-4'}$ = 4.2 Hz, 1, H-3'), 4.52 ("t", $J_{2'-3'}$ = 5.0 Hz, $J_{2'-1'}$ = 5.0 Hz, 1, H-2'), 4.7 (br, 4, 4 x OH) 5.95 (d, $J_{1'-2'}$ = 5.0 Hz, 1, H-1'), 6.95-7.3 (m, 5, C_6H_5), 8.58 (s, 1, H-2), 8.69 (s, 1, H-8), 9.7 (br, 1, NH); ms m/z (glycerol/FAB) = 424 (MH^+); Anal. ($C_{16}H_{18}N_5O_7P\cdot0.25\ H_2O$) Calc: C, 44.45; H, 4.43; N, 16.20. Found: C, 44.32; H, 4.40; N, 15.95.

2',3',5'-Tri-O-acetyl-6-azido-9-β-D-ribofuranosylpurine (91)

To a suspension of 250 mg (0.85 mmol) of 6-azido-9-β-D-ribofuranosylpurine (90) in 2.5 mL of Ac_2O, 6 mL of dry pyridine was added dropwise with stirring and the resulting reaction mixture stirred overnight at room temperature. Evaporation of solvents followed by co-evaporation of the residue with toluene gave a foam which was recrystallized from $CHCl_3$ by diffusion of Et_2O to afford 325 mg (91%) of (91): mp 62-64°C; UV (MeOH) max

286 nm, min 237 nm; ^{1}H NMR (200 MHz) δ 2.04, 2.12 (s, "s", 3, 6, 3 x OAc), 4.37 (m, 3, H-4', H-5', H-5"), 5.62 ("t", $J_{3'-4'}$ = 5.5 Hz, $J_{3'-2'}$ = 5.5 Hz, 1, H-3'), 5.97 ("t", $J_{2'-3'}$ = 5.5 Hz, $J_{2'-1'}$ = 5.2 Hz, 1, H-2'), 6.46 (d, $J_{1'-2'}$ = 5.2 Hz, 1, H-1'), 8.88 (s, 1, H-2), 10.18 (s, 1, H-8); MS m/z 419.1189 (3.5%, $M^+[C_{16}H_{17}N_7O_7]$ = 419.1187), 259.0816 (85.7%, sugar), 133.0392 (3.5%, BHCH=CH_2-N_2), 119.0358 (1.2%, BH-N_3); Anal. ($C_{16}H_{17}N_7O_7$) Calc: C, 45.83; H, 4.09; N, 23.38. Found: C, 45.58; H, 4.10; N, 22.99.

Reaction of (91) with triphenyl phosphite to give 2',3',5'-tri-O-acetyl-6-N-(diphenylphosphoryl)adenosine (58)

An 83.6 mg (0.2 mmol) sample of (91) dissolved in 10 mL of dry dioxane was refluxed with 262 μl (1 mmol) of triphenyl phosphite for 4 h. Evaporation of volatile solvents and chromatography on silica gel# using 2% MeOH/$CHCl_3$ as eluant gave 63 mg (78%) of (58): mp 69-71°C. The physical properties of (58) were identical with the previous data (for 65 → 79 → 58).

Triethyl N-[9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-9H-purin-6-yl]phosphorimidate (92)

A 41.9 mg (0.1 mmol) sample of (91) dissolved in 5 mL of dry dioxane was stirred with 86 μl (0.5 mmol) of triethyl phosphite at room temperature for 30 min. Evaporation of volatile solvents and chromatography on silica gel# with 2% MeOH/$CHCl_3$ as eluant provided 54 mg (96%) of (92) as a thick syrup: UV (MeOH) max 266 nm (ε 19,100), min 229 nm (ε 3,600); (H^+) max 266 nm (ε 18,400), min 230 nm (ε 4,000); (OH^-) max 267 nm (ε 18,300), min 234 nm (ε 4,500); ^1H NMR (200 MHz) δ 1.3 (m, 9, 3 x $-CH_2\underline{CH_3}$), 2.04, 2.08, 2.14 (3 s, 3 x 3, 3 x OAc), 4.22 (m, 9, H-4', H-5', H-5", 3 x $-\underline{CH_2}CH_3$), 5.66 ("t", $J_{3'-4'}$ = 5.2 Hz, $J_{3'-2'}$ = 5.6 Hz, 1, H-3'), 6.08 ("t", $J_{2'-3"}$ = 5.6 Hz, $J_{2'-1'}$ = 5.4 Hz, 1, H-2'), 6.23 (d, $J_{1'-2'}$ = 5.4 Hz, 1, H-1'), 8.3 (s, 1, H-2), 8.35 (s, 1, H-8); MS m/z (glycerol/FAB) = 558 (MH^+).

2',3',5'-Tri-O-acetyl-6-N-(diethylphosphoryl)adenosine (93)

A 54 mg (0.096 mmol) sample of (92) dissolved in 1.5 mL of 95% EtOH was stirred with 3 mL of 0.01 N HCl/H_2O for 4 days at room temperature. The reaction mixture was evaporated to dryness and the residue chromatographed by PLC to afford 26 mg (51%) of (93): mp 34-36°C; UV (MeOH) max 261 nm (ε 14,800), min 233 nm (ε 1,800); (H^+) max 261 nm (ε 15,000), min 234 nm (ε 4,800); (OH^-) max 275 nm (ε 21,400), min 238 nm (ε 1,900); ^1H NMR (200 MHz) δ 1.23 (t, J = 6.5 Hz, 6, 2 x $-CH_2\underline{CH_3}$), 2.02, 2.04, 2.12 (3 s, 3 x 3, 3 x OAc), 4.02 (m, 4, 2 x $-\underline{CH_2}CH_3$), 4.32 (m, 3, H-4', H-5', H-5"), 5.64 ("t", $J_{3'-2'}$ = 5.0 Hz, $J_{3'-4'}$ = 5.6 Hz, 1, H-3'), 6.04 ("t", $J_{2'-3'}$ = 5.0 Hz, $J_{2'-1'}$ = 5.8 Hz, 1, H-2'), 6.24 (d, $J_{1'-2'}$ = 5.8 Hz, 1, H-1'), 8.4 (s, 1, H-2), 8.48 (s, 1, H-8), 9.62 (br, 1, NH); MS m/z (glycerol/FAB) = 530 (MH^+).

6-*N*-(Diethylphosphoryl)adenosine (94)

Procedure C was applied to 200 mg (0.378 mmol) of (93) using 1 mL of dioxane and 2.5 mL of conc. aq. NH_3. The residue after evaporation of volatiles was chromatographed on silica gel# using 1% MeOH/$CHCl_3$ as eluant and recrystallized from $CHCl_3$ by diffusion of Et_2O to give 140 mg (70%) of (94): mp 59-61°C; UV (MeOH) max 260 nm (ε 14,600), min 226 nm (ε 1,700); (H^+) max 260 nm (ε 15,000), min 228 nm (ε 5,000); (OH^-) max 276 nm (ε 21,400), min 238 nm (ε 1,900); ^1H NMR δ 1.23 (m, 6, 2 x CH_3), 3.1-4.2 (m, 8, 2 x CH_2, H-3', H-4', H-5', H-5"), 4.6 (m, 1, H-2'), 5.2 (m, 2, OH-3', OH-5'), 5.51 (d, $J_{OH-2'}$ = 5.8 Hz, OH-2'), 5.8 (d, $J_{1'-2'}$ = 6.0 Hz, 1, H-1'), 8.46 (s, 1, H-2), 8.61 (s, 1, H-8), 9.54 (br, 1, NH); MS m/z (glycerol-sulfolane/FAB) = 404 (MH^+); Anal. ($C_{14}H_{22}N_5O_7P \cdot 0.25\ H_2O$) Calc: C, 41.23; H, 5.56; N, 17.17. Found: C, 41.27; H, 5.44; N, 16.83.

Reaction of 2,3,4,6-tetra-O-benzyl-α-D-glucopyranose (95) with diethyl phosphorochloridite and (91)

A 108 mg (0.2 mmol) sample of (95) was dissolved in 5 mL of dry THF and cooled to -78°C. Addition of 70 μl (0.4 mmol) of iPr_2NEt and 45 μl (0.31 mmol) of diethyl phosphorochloridite was followed by stirring for 45 min at -78°C. A 41.9 mg (0.1 mmol) sample of the azido nucleoside (91) was added and the solution allowed to warm gradually. Dry dioxane (10 mL) was added at 0°C and the reaction mixture stirred at room temperature overnight. Evaporation of volatile liquids and chromatography of the residue on silica gel# with 1% MeOH/$CHCl_3$ gave 33 mg (62%) of 2',3',5'-tri-O-acetyl-6-N-(diethylphosphoryl)adenosine (93). The physical properties of this compound were identical with the previous data (for 91 → 92 → 93).

Reaction of (95) with diphenyl phosphorochloridite and (91)

The preceeding procedure was followed with 108 mg (0.2 mmol) of (95) using 17.4 μl (0.24 mmol) of diphenyl phosphorochloridite. This after chromatography afforded 15 mg (48%) of 2',3',5'-tri-O-acetyl-6-N-(diphenylphosphoryl)adenosine (58): mp 69-71°C. The physical properties of this compound were identical with the previous data (for 65 → 58).

2',3',5'-Tri-O-acetyl-6-N-[ethyl(methyl 2,3,4-tri-O-benzyl-α-D-glucopyranosid-6-yl)phosphoryl]adenosine (100)

A 46.4 mg (0.1 mmol) sample of methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (98) dissolved in 5 mL of dry THF was cooled to -78 C. Addition of 34.8 μl (0.2 mmol) of iPr_2NEt and 43.5 μl (0.3 mmol) of diethylphosphorochloridite was followed by stirring for 1 h at -78°C for 1 h. A 20.9 mg (0.05 mmol) sample of nucleoside (91) was added and the reaction mixture gradually allowed to warm up. At 0°C, 5 mL of dry dioxane was added and the resulting solution stirred overnight at room temperature. Evaporation of volatile solvents and chromatography on silica gel[#] using 1% MeOH/$CHCl_3$ as eluant gave 34 mg (72%) of (100): UV (MeOH) max 258 nm, min 227 nm; (H^+) max 259 nm, min 228 nm; (OH^-) max 277 nm, min 236 nm; ^1H NMR (200 MHz) δ 1.2 (m, 3, $-CH_2\underline{CH_3}$), 2.0, 2.02, 2.12 (3 s, 3 x 3, 3 x OAc), 3.33-4.9 (m, 21), 5.64 ("t", $J_{3'-4'}$ = 4.5 Hz, $J_{3'-2'}$ = 5.8 Hz, 1, H-3'), 6.04 ("t", $J_{2'-3'}$ = 5.8 Hz, $J_{2'-1'}$ = 5.0 Hz, 1, H-2'), 6.28 (d, $J_{1'-2'}$ = 5.0 Hz, 1, H-1'), 7.2-7.5 (m, 15, 3 x C_6H_5), 8.4 (s, 1, H-2), 8.66 (s, 1, H-8), 10.82 (br, 1, NH); MS m/z (glycerol-sulfolane/FAB) = 948 (MH^+).

6-N-[Ethyl(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosid-6-yl)phosphoryl]adenosine (101)

Procedure C was applied to 61 mg (0.064 mmol) of (100) using 800 μl of dioxane and 3 mL of conc. aq. NH_3, to give 45 mg (85%) of (101) after recrystallization from $CHCl_3$ by diffusion of Et_2O: mp 82-84°C; UV (MeOH) max 260 nm (ε 14,400), min 226 nm (ε 1,800); (H^+) max 261 nm (ε 15,300), min 230 nm (ε 9,700); (OH^-) max 277 nm (ε 21,000), min 236 nm (ε 13,900); ^1H NMR (200 MHz) δ 1.2 (t, J = 7.0 Hz, 3, $-CH_2\underline{CH_3}$), 3.3 (s, 3, $-OCH_3$), 3.4-3.84 (m, 6), 3.9-3.42 (m, 6), 4.52-4.92 (m, 8), 5.2 (t, $J_{OH-5,5''}$ = 5.2 Hz, 1, OH-5'), 5.25 (d, $J_{OH-3'}$ = 5.0 Hz, 1, OH-3'), 5.51 (d, $J_{OH-2'}$ = 5.8 Hz, 1, OH-2'), 5.98 (d,

$J_{1'-2'}$ = 6.0 Hz, 1, H-1'), 7.2-7.5 (m, 15, 3 x C_6H_5), 8.42 (s, 1, H-2), 8.62 (s, 1, H-8), 9.66 (br, 1, NH); MS m/z (glycerol-sulfolane/FAB) = 822 (MH^+); Anal. ($C_{40}H_{48}N_5O_{12}P\cdot H_2O$) Calc: C, 57.21; H, 6.00; N, 8.34. Found: C, 57.24; H, 5.77; N, 8.49.

5'-Deoxy-5'-*N*-(diethylphosphoryl)amino-2',3'-*O*-isopropylideneadenosine (113)

To a sample of 108 mg (0.3 mmol) of 5'-azido-5'-deoxy-6-*N*-formyl-2',3'-*O*-isopropylideneadenosine (112) in 15 mL of dry dioxane was added 210 µl (1.5 mmol) of triethyl phosphite and the resulting solution refluxed for 2 h. Evaporation of volatile solvents gave a residue which was chromatographed on silica gel# using 5% MeOH/$CHCl_3$ as the eluant. Evaporation of appropriate fractions and recrystallization of the resulting solid from $CHCl_3$ by diffusion of Et_2O gave 102 mg (77%) of (113): mp 194-196°C; UV (MeOH) max 260 nm (ε 15,600), min 225 nm (ε 2,000); (H^+) max 256 nm (ε 16,000), min 227 nm (ε 3,500); (OH^-) max 259 nm (ε 15,900), min 234 nm (ε 5,900); ^1H NMR (200 MHz) δ 1.15 (q*, 6, 2 x CH_3), 1.35, 1.57 (2 s, 2 x 3, $-CMe_2$), 3.02 (m, 2, H-5', H-5"), 3.88

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* Overlapping triplets.

(m, 4, 2 x $-CH_2$), 4.18 (m, $J_{4'-3'}$ = 2.5 Hz, 1, H-4'), 5.04 (dd, $J_{3'-4'}$ = 2.5 Hz, $J_{3'-2'}$ = 2.5 Hz, 1, H-3'), 5.35 (m, 1, NH), 6.13 (d, $J_{1'-2'}$ = 3.0 Hz, 1, H-1'), 7.4 (br s, 1, NH_2), 8.16 (s, 1, H-2), 8.35 (s, 1, H-8); MS m/z 442.1730 (2.6%, $M^+[C_{17}H_{27}N_6O_6P]$ = 442.1734), 164.0569 (100%, BH-CHO), 162.0772 (4.4%, $BHCH=CH_2$), 136.0630 (90.1%, B + 2H), 135.0553 (40.2%, B + H); Anal. ($C_{17}H_{27}N_6O_6P$) Calc: C, 46.15; H, 6.15; N, 19.00. Found: C, 46.03; H, 6.11; N, 18.90.

5'-Deoxy-5'-N-(diphenylphosphoryl)amino-2',3'-O-isopropylideneadenosine (114)

A 72 mg (0.2 mmol) sample of (112) in 10 mL of dry dioxane was refluxed with 262 μl (1 mmol) of triphenyl phosphite for 4 h. Evaporation of volatile solvents and chromatography of the resulting residue on silica gel* using 5% $MeOH/CHCl_3$ as eluant afforded a solid which was recrystallized from $CHCl_3$ by diffusion of Et_2O, to give 60 mg (57%) of (114): mp 154-155°C; UV (MeOH) max 261 nm (ε 15,200); min 225 nm (ε 1,470); (H^+) max 257 nm (ε 15,100), min 229 nm (ε 4,900); (OH^-) max 259 nm (ε 15,300), min 246 nm (ε 12,600); ^1H NMR (200 MHz) δ 1.2, 1.54 (2 s, 2 x 3, $-CMe_2$), 3.22 (m, 2, H-5', H-5"), 4.17 (m, 1, H-4'), 4.78 (dd, $J_{3'-4'}$ = 2.6 Hz, $J_{3'-2'}$ = 2.6 Hz, 1, H-3'), 5.23 (dd, $J_{2'-3'}$ = 2.6 Hz, $J_{2'-1'}$ = 3.4 Hz, 1, H-2'), 6.05 (d, $J_{1'-2'}$ = 3.4 Hz, 1, H-1'), 6.5 (m, 1, NH), 7.1-7.5 (m, 12, 2 x C_6H_5, NH_2), 8.08 (s, 1, H-2), 8.3 (s, 1, H-8); MS m/z 538.1729 (1.1%, $M^+[C_{25}H_{27}N_6O_6P]$ = 538.1710), 523.1489 (2.4%, M^+-CH_3), 445.1380 (1.0%, $M^+-C_6H_5O$), 164.0572 (100%, BH-CHO), 162.0772 (1.5%, $BHCH{=}CH_2$), 136.0624 (56.8%, B + 2H), 135.0547 (24.1%, B + H); Anal. ($C_{25}H_{27}N_6O_6P$). Calc: C, 55.76; H, 5.05; N, 15.61. Found: C, 56.00; H, 5.27; N, 15.38.

N-Octanoyl-tri-O-ethylphosphorimidate (144)

To a solution of ~500 mg (2.9 mmol) of octanoyl azide (143) in ~6 mL of Et_2O (at 0°C) was added 2.5 mL (14.5 mmol) of triethyl phosphite and the resulting reaction mixture refluxed for 90 min. After removal of the volatile solvents, the residue was partitioned between Et_2O and H_2O. The organic layer was washed successively with H_2O, sat. NaCl/H_2O, dried over Na_2SO_4 and evaporated to give 895 mg (98%) of (144) as an oil: IR (neat): ν (P=N) 1380 cm^{-1}, ν (C=O) 1620 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz), δ 0.9 (t, J = 6.0 Hz, 3, $-CH_2\underline{CH_3}$), 1.3-1.8 (m, 19, 5 x CH_2, 3 x $-OCH_2\underline{CH_3}$), 2.33 (t, 2, $-C(O)CH_2$), 4.2 (m, 6, 3 x $-\underline{OCH_2}CH_3$); MS m/z 309.1913 (3.7%, $M^+[C_{14}H_{30}NO_4P]$ = 309.1915), 208.0744 (89.8%, $M^+-CH_3(CH_2)_6-$), 155.0479

(57.2%, $(EtO)_2P^+(OH)_2$), 127.1121 (1.5%, $CH_3(CH_2)_6C{\equiv}O^+$); Anal. ($C_{14}H_{30}NO_4P\cdot 0.25\ H_2O$) Calc: C, 53.92; H, 9.86; N, 4.22. Found: C, 53.66; H, 9.62; N, 4.49.

N-Octanoyl-di-*O*-ethylphosphoramidate (145)

To a 1 g (3.25 mmol) sample of (144) dissolved in 5 mL of abs. EtOH was added 5 mL of 0.01 N HCl/H_2O and the resulting solution stirred overnight at room temperature. Evaporation of solvents gave a residue which was extracted into Et_2O. The ether layer was washed successively with sat. $NaHCO_3/H_2O$, H_2O, sat. $NaCl/H_2O$, dried over Na_2SO_4 and evaporated to give 854 mg (94%) of (145) as a greasy solid: IR (neat): ν (C=O) 1680 cm^{-1}, ν (P=O) 1240 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 0.89 (t, J = 6 Hz, 3, $-CH_2\underline{CH_3}$), 1.2-1.8 (m, 16, 5 x CH_2, 2 x $-OCH_2\underline{CH_3}$), 2.29 (m, 2, $-C(O)CH_2$), 4.14 (m, 4, 2 x $-O\underline{CH_2}CH_3$), 5.6 (br, 1, NH); MS m/z 279.1599 (2.1%, $M^+[C_{12}H_{26}NO_4P]$ = 279.1598), 181.0620 (9.4%, $(EtO)_2P(O)NHCO^+$), 155.0467 (100%, $(EtO)_2PO_2H+1$), 126.1281 (1.2%, $CH_3(CH_2)_6C{\equiv}NH^+$).

5'-*O*-(Diphenylphosphityl)-2',3'-*O*-isopropylidene-adenosine (147b)

A 307 mg (1 mmol) sample of 2',3'-*O*-isopropylideneadenosine (146) was suspended in 10 mL of dry CH_2Cl_2. Dry pyridine (170 µl, 2 mmol) and 172 µl (1.2 mmol) of diphenyl phosphorochloridite were added and the reaction mixture stirred at room temperature for 1 h. Water (2 mL) was added and the reaction mixture evaporated to dryness. The residue was coevaporated with toluene several times, extracted into $CHCl_3$, and the organic layer washed successively with 2% aq. AcOH, sat. $NaHCO_3/H_2O$, H_2O, sat. $NaCl/H_2O$, dried over Na_2SO_4 and evaporated. The solid residue was chromatographed on silica gel using 1% $MeOH/CHCl_3$ as eluant. Evaporation of appropriate fractions afforded 392 mg (75%) of (147b) which was recrystallized from EtOH by diffusion of Et_2O: mp 78-80°C; λ_{max}^{MeOH} = 258 nm; ^1H NMR (200 MHz) δ 1.34, 1.56 (2 s, 2 x 3, $-CMe_2$), 4.34 (m, 3, H-4', H-5', H-5"), 5.1 (dd, $J_{3'-4'}$ = 5.8 Hz, $J_{3'-2'}$ = 5.0 Hz, 1, H-3'), 5.5 (dd, $J_{2'-1'}$ = 4.0 Hz, $J_{2'-3'}$ = 5.0 Hz, 1, H-2'), 6.23 (d, $J_{1'-2'}$ = 4.0 Hz, 1, H-1'), 6.95-7.4 (m, 12, 2 x C_6H_5, NH_2), 8.12 (s, 1, H-2), 8.32 (s, 1, H-8); MS m/z 430.1275 (100%, $M^+-C_6H_5O$ [$C_{19}H_{21}N_5O_5P$] = 430.1279), 164.0572 (3.2%, BH-CHO), 162.0776 (0.8%, $BHCH=CH_2$) 136.0622 (21.5%, B + 2H), 135.0546 (6.7%, B + H).

5'-O-(Phenylphosphoramidatyl)-2',3'-O-isopropylidene-adenosine (148)

A 104.6 mg (0.2 mmol) sample of (147b) was dissolved in 10 mL of dry dioxane. A solution of ~169 mg (~1 mmol) of octanoyl azide in 10 mL of Et_2O was added to the above solution and the reaction mixture refluxed for ~10 h. Evaporation of volatile solvents and chromatography on silica gel using 2% $MeOH/CHCl_3$ as eluant yielded 20 mg (32%) of (146) which was recrystallized from H_2O: mp 216-217°C. This compound was found to be identical with 2',3'-O-isopropylideneadenosine by the usual criteria. Further elution of the column gave 60 mg (65%) of (148) which was recrystallized from $CHCl_3$ by diffusion of Et_2O: mp 90-92°C; UV (MeOH) max 259 nm (ε 15,400), min 225 (ε 2,100); (H^+) max 257 nm (ε 13,900), min 229 nm (ε 3,100); (OH^-) max 259 nm (ε 16,000); ^1H NMR (200 MHz) δ 1.34, 1.55 (2 s, 2 x 3, $-CMe_2$) 4.3 (m, 3, H-2', H-3', H-4'), 5.05 (m, 1, H-5'), 5.17 (d, J_{H-N-P} = 6.5 Hz, 2, NH_2), 5.4 (m, 1, H-5"), 6.21 (d, $J_{1'-2'}$ = 2.5 Hz, 1, H-1'), 7.1-7.5 (m, 7, C_6H_5, 6-NH_2), 8.16 (s, 1, H-2), 8.33 (d, 1, H-8), MS m/z (glycerol/FAB) = 463 (MH^+); Anal. ($C_{19}H_{23}N_6O_6P$) Calc: C, 49.35; H, 5.01; N, 18.18. Found: C, 48.94; H, 5.07; N, 18.16.

6-N-Benzoyl-2',3'-O-isopropylideneadenosine (150)

A 307 mg (1 mmol) sample of 2',3'-O-isopropylideneadenosine (146) was converted to the title compound using identical conditions as described for 2'-deoxyadenosine.[205] This gave 378 mg (92%) of (150): mp 138-140°C; UV (MeOH) max 280 nm (ϵ 20,100), min 243 nm (ϵ 7,000); ^{1}H NMR (200 MHz) δ 1.38, 1.56 (2 s, 2 x 3, -CMe_2), 3.56 (m, 2, H-5', H-5"), 4.29 (m, 1, H-4'), 5.02 (dd, 1, H-3'), 5.15 (t, 1, OH), 5.44 (dd, 1, H-2'), 6.58 (d, 1, H-1'), 7.5-7.8 and 8.0-8.1 (m, 5, C_6H_5), 8.67 (s, 1, H-2), 8.76 (s, 1, H-8), 10.2 (br s, 1, NH); MS m/z 411.1553 (8.1%, $M^+[C_{20}H_{21}N_5O_5]$ = 411.1543), 381. 1431 (2.6%, M^+-CH_2O), 164.0572 (18.9%, BH-CHO); Anal. ($C_{20}H_{21}N_5O_5$) Calc: C, 58.39; H, 5.15; N, 17.02. Found: C, 58.09; H, 5.06; N, 16.92.

6-N-Benzoyl-5'-O-[(N-benzoyl)phosphoramidatyl]-2',3'-O-isopropylideneadenosine (152)

To a sample of 411 mg (1 mmol) of (150) in 15 mL of dry pyridine, was added 238 mg (1 mmol) of N-benzoyl phosphoramidodichloridate and the reaction mixture stirred at room temperature for 1 h. Evaporation of pyridine and coevaporation of the residue with toluene gave a solid which was chromatographed on silica gel‡. The column was eluted with 4% MeOH/$CHCl_3$, the eluate concentrated and the product recrystallized from $CHCl_3$ by diffusion of Et_2O to afford 50 mg (5%) of the bis-product (151): mp 135-137°C; UV (MeOH) max 230 nm (ε 37,200) and 280 nm (ε 36,000), min 253 nm (ε 22,000); (H^+) max 289 nm (ε 38,000), min 264 nm (ε 22,000); (OH^-) max 305 nm (ε 21,800), min 262 nm (ε 15,200); ^1H NMR (200 MHz) δ 1.29, 1.54 (2 "s", 2 x 6, 2 x $-CMe_2$) 4.28 (m, 4, 2 x H-5', H-5"), 4.45 (m, 2, 2 x H-4'), 5.1 (m, 2, 2 x H-3'), 5.44 (m, 2, 2 x H-2'), 6.31 ("t", $J_{1'-2'}$ = 2.2 Hz, 2, 2 x H-1'), 7.5-8.1 (m, 15, 3 x C_6H_5), 8.61, 8.63, 8.68, 8.71 (4 s, 4, 2 x H-2, 2 x H-8), 10.12 ("s", 2, 2 x 6-NH), 11.21 (br, 1, NH) MS m/z (glycerol/FAB) = 988 (MH^+); Anal. ($C_{37}H_{46}N_{11}O_{12}P\cdot 2H_2O$) Calc: C, 55.13; H, 4.92; N, 14.74. Found: C, 54.93; H, 4.80; N, 15.05.

When the column was further eluted with 20% MeOH/$CHCl_3$ 260 mg of product containing (152) was obtained. However pure (152) was not obtained; ^1H NMR (400 MHz): δ 1.3, 1.53 (2 s, 2 x 3, $-CMe_2$), 4.0 (m, 2, H-5', H-5"), 4.38 (m, 1, H-4'), 5.06 (m, 1, H-3'), 5.48 (m, 1, H-2'), 6.32 (d, 1, H-1'), 7.44-8.2 (m, 10, 2 x C_6H_5), 9.64 (br s, 1, 6-NH), 11.21 (br, 2, NH, OH); MS m/z (glycerol/FAB) = 595 (MH^+).

DL-threo-2,3-di-*O*-benzyl-4-methylpentanamide (153)

A 147 mg (1 mmol) sample of (7') was dissolved in 10 mL of dry DMF. This solution was cooled to -23°C and treated with 108 mg (4.69 mmol) of sodium hydride (50% dispersion in oil was washed with dry benzene under an atmosphere of nitrogen and transferred to the above solution in 1.5 mL of dry DMF at -23°C). The reaction mixture was stirred at -23°C for 20 min and treated with 500 μl (4.2 mmol) of benzyl bromide and further stirred for 2 h at -23°C. Methanol (1 mL) was added and the solution gradually warmed up. It was evaporated to dryness and the residue partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with sat. $NaCl/H_2O$, dried over Na_2SO_4 and evaporated to give an oil which was chromatographed on silica gel using 3% $MeOH/CHCl_3$ as eluant. Evaporation of appropriate fractions gave 196 mg (60%) of (153): mp 118-120°C; ^1H NMR (200 MHz) δ 0.8, 0.92 (2 d, $J = 7.0$ Hz, 2 x 3, $-CH\underline{Me_2}$), 1.86 (m, 1, H-4 $[-C\underline{H}Me_2]$), 3.45 (dd, 1, H-3), 3.85 (d, $J_{2-3} = 4.0$ Hz, 1, H-2), 4.4 (dd, $J_{gem} = 11.0$ Hz, 2, $-\underline{CH_2}Ph$), 4.66 (dd, $J_{gem} = 10.5$ Hz, 2, $-\underline{CH_2}Ph$), 7.2-7.5 (m, 12, 2 x C_6H_5, NH_2); MS m/z 236.1288 (0.1%, $M^+-C_7H_7$), 91.0535 (100%, C_7H_7); MS m/z (glycerol/FAB) = 328 (MH^+); Anal. ($C_{20}H_{25}NO_3$) Calc: C, 73.36; H, 7.70; N, 4.28. Found: C, 73.35; H, 7.65; N, 4.33.

Bis(6-N-benzoyl-2',3'-O-isopropylideneadenosin-5'-yl) 2-chlorophenyl phosphate (156)

To a 78 mg (0.19 mmol) sample of (150) in 1 mL of dry THF at -78°C, were added 18 μl (0.21 mmol) of pyridine and 17 μl (0.11 mmol) of 2-chlorophenyl phosphorodichloridite. A white precipitate was formed. The mixture was stirred at -78°C for 30 min and gradually warmed up to ~-10°C, whereupon a solution of iodine (27 mg, 0.11 mmol) in 3 mL THF-H_2O (2:1) was added. The reaction mixture was warmed up to room temperature. Volatile solvents were removed *in vacuo*. The residual gum was taken up in 2 mL of 10% 1-butanol in $CHCl_3$ and washed with freshly prepared 5% $NaHSO_3/H_2O$. The organic layer was washed with 1 mL portion of H_2O a few times, dried over Na_2SO_4 and evaporated to give a gum. Chromatography of this residue on silica gel using 2% MeOH/$CHCl_3$ afforded 147 mg (78%) of (156) from $CHCl_3$ by diffusion of Et_2O: mp 104-105°C; ^1H NMR (200 MHz) δ 1.3, 1.54 (2 "s", 2 x 6, 2 x -CMe_2), 4.4 (m, 6, 2 x H-4', H-5', H-5"), 5.05 (m, 2, 2 x H-3'), 5.45 (m, 2, 2 x H-2'), 6.34 ("d",* 2, 2 x H-1'), 7.08-8.1 (m, 14, C_6H_5's), 11.23 (br "s", 2, 2 x NH); MS m/z (glycerol/FAB) = 995 (MH^+); Anal. ($C_{46}H_{44}N_{10}O_{12}PCl \cdot H_2O$): C, 54.52; H, 4.58; N, 13.82. Found: C, 54.35; H, 4.33; N, 13.54.

(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl)methyl iodide (160)

To a sample of 1.75 g (2.26 mmol) of (2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)methylmercury chloride (159) in 17.5 mL of CH_2Cl_2 was added a solution of 626 mg (2.46 mmol) of iodine in 31 mL CH_2Cl_2. The reaction mixture was stirred at room temperature for 1.5 h. It was partitioned between CH_2Cl_2 and H_2O and the organic layer washed successively with 10% aq. $NaHSO_3$, 5% aq. KI, H_2O, sat. aq. NaCl, dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed on silica gel and product eluted with 1% MeOH/$CHCl_3$. Evaporation of appropriate fractions yielded 1.28 g (85%) of (160): mp 79-81°C; $[\alpha]_D^{20} = +51°$ (c = 0.56, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz) δ 3.3-4.2 (m, 9, CH_2I, H-1, H-2, H-3, H-4, H-5, H-6, H-6'), 4.4-4.9 (m, 8, 4 x $-\underline{CH_2}Ph$), 7.12-7.4 (m, 20, 4 x C_6H_5); MS m/z 573.1136 (1.5%, $M^+-C_7H_7[C_{28}H_{30}O_5I]$ =

* "d" = overlapping doublets.

573.1139), 91.0549 (100%, C_7H_7); Anal. ($C_{35}H_{37}O_5I$) Calc: C, 63.26; H, 5.61. Found: C, 63.53; H, 5.77.

Diethyl (2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)methylphosphonate (161a)

A 325 mg (0.49 mmol) sample of (160) was refluxed under argon for 4 h in 4 mL of triethyl phosphite. The excess of the reagent was removed under reduced pressure and the residue chromatographed on silica gel using 1% $MeOH/CHCl_3$ as eluant to yield 310 mg (94%) of (161a) as an oil: ^1H NMR (C_6D_6, 400 MHz) was identical with the published data.[211] ^1H NMR ($CDCl_3$, 200 MHz) δ 1.27 (m, J = 7.0 Hz, 6, 2 x $-CH_2\underline{CH_3}$), 2.12-2.13 (m, 2, H-1'a, H-1'b), 3.6-3.84 (m, 6), 4.1 (m, J = 7.0 Hz, 4, 2 x $-\underline{CH_2}CH_3$), 4.42-4.95 (m, 10), 7.1-7.42 (m, 20, 4 x C_6H_5); MS m/z (0.4%, M^+-3 x C_7H_7O), 264.1014 (0.9%, M^+-4 x C_7H_7O), 91.054 (100%, C_7H_7); MS m/z (glycerol/FAB) = 675 (MH^+).

Dimethyl (2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-methylphosphonate (161b)

A 325 mg (0.049 mmol) sample of (160) was refluxed under argon for 10 h in 8 mL of trimethyl phosphite. The excess of the solvent was removed under reduced pressure and the residue chromatographed on silica gel using 1% MeOH/$CHCl_3$ to afford 148 mg (47%) of (161b) as an oil: $[\alpha]_D^{20} = -30°$ (c = 0.66, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz) δ 2.14-2.9 (m, 2, H-1'a, H-1'b), 3.6-3.8 (m, 12), 4.43-4.91 (m, 9), 7.1-7.35 (m, 20, 4 x C_6H_5); MS m/z 646.2696 (0.2%, $M^+[C_{37}H_{43}O_8P]$ = 646.2697), 540.2279 (0.2%, $M^+-C_7H_7$), 449.1720 (0.4%, $M^+-2 \times C_7H_7$), 91.0542 (100%, C_7H_7); Anal. ($C_{37}H_{43}O_8P$) Calc: C, 68.72; H, 6.70. Found: C, 68.09; H, 6.66..

3,4,6,7-Tetra-O-benzyl-1,2-dideoxy-D-gluco-hept-1-enitol (162)

A sample of 1.08 g (2 mmol) of (47) in 6 mL of THF-HMPA (4:1) was treated with 1.32 mL of 1.3 M n-BuLi/hexane at 0°C. A solution of methylenetriphenylphosphorane in 4 mL of THF-HMPA (4:1), prepared at -15°C under argon from 1.43 g (4 mmol) of $Ph_3P^+CH_3Br^-$ and 3.81 mL of 1.3 M n-BuLi/hexane was added to the above solution at room temperature. This reaction mixture was stirred under argon at room temperature for 3 h. It was evaporated to dryness and the residue dissolved in ~25 mL CH_2Cl_2. The CH_2Cl_2 layer was washed successively with sat. $NaHCO_3/H_2O$, H_2O, sat. $NaCl/H_2O$, dried over Na_2SO_4 and evaporated to give a dark colored oil. This was chromatographed on a silica gel column using 0.5% $MeOH/CHCl_3$ as eluant. Evaporation of appropriate fractions yielded 805 mg (74%) of (162) as an oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 2.56 (d, J_{OH-5} = 8.5 Hz, 1, OH), 3.58-3.9 (m, 5), 3.74 (m, 1, H-5), 4.13 ("t", J_{3-4} = 7.5 Hz, J_{3-2} = 7.5 Hz, 1, H-3), 4.32-4.93 (m, 8), 5.35 (m, J_{gem} = 1.75 Hz, 2, 2 x H-1), 5.85 (m, $J_{2-pro\ E}$ = 9.5 Hz, $J_{2-pro\ Z}$ 17.5 Hz, J_{2-3} = 7.5 Hz, 1, H-2), 7.2-7.4 (m, 20, 4 x C_6H_5); MS m/z 447.2184 (0.2%, $M^+-C_7H_7[C_{28}H_{31}O_5]$ = 447.2183), 91.0530 (100%, C_7H_7); Anal. ($C_{35}H_{38}O_5$) Calc: C, 78.04; H, 7.11. Found: C, 77.83; H, 7.07.

(2,3,5,6-Tetra-O-benzyl-α-D-glucofuranosyl)methylmercury chloride (163)

A 780 mg (1.4 mmol) sample of (162) in 7.8 mL of dry THF was stirred for 2 h at room temperature with 461 mg (1.44 mmol) of $Hg(OAc)_2$. A solution of 178 mg (2.38 mmol) of KCl in 535 μl of H_2O was added and the reaction mixture stirred for 1 h. Chloroform (20 mL) was added and the organic layer washed with H_2O, sat. $NaCl/H_2O$, dried over Na_2SO_4 and evaporated. The residue was chromatographed on a column of silica gel using 1% $MeOH/CHCl_3$ to afford 873 mg (78%) of (163) as an oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 1.74 (dd, $J_{1'b-1'a}$ = 12.5 Hz, $J_{1'b-1}$ = 3.5 Hz, 1,

H-1'b), 2.1 ($J_{1'a-1'b}$ = 12.5 Hz, $J_{1'a-1}$ = 6.0 Hz, 1, H-1'a), 3.7 (m, 2, H-2, H-6'), 3.91 (dd, $J_{6-6'}$ = 11.0 Hz, J_{6-5} = 2.0 Hz, 1, H-6), 4.0 (m, 1, H-5), 4.18 (d, J_{3-4} = 4.0 Hz, 1, H-3), 4.28 (dd, J_{4-3} = 4.0 Hz, J_{4-5} = 9.5 Hz, 1, H-4), 4.33-4.88 (m, 8, 4 x -$\underline{CH_2}$Ph), 4.67 (m, 1, H-1), 7.24-7.44 (m, 20, 4 x C_6H_5); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 28.74 (CH_2HgCl); MS m/z (glycerol/FAB) = 775 (MH^+ for ^{202}Hg, ^{35}Cl); Anal. ($C_{35}H_{37}ClHgO_5$) Calc: C, 54.33; H, 4.82. Found: C, 54.41; H, 4.89.

(2,3,5,6-Tetra-O-benzyl-α-D-glucofuranosyl)methyl iodide (164)

To a sample of 800 mg (1.035 mmol) of (163) in 8 mL of CH_2Cl_2 was added a solution of 286 mg (1.125 mmol) of iodine in 7 mL of CH_2Cl_2. After stirring the reaction mixture at r.t. for ~2 h, the CH_2Cl_2 layer was washed with 10% aq. $NaHSO_3$, 5% aq. KI, H_2O, sat. aq. NaCl, dried over Na_2SO_4 and evaporated to give a residue which was subjected to chromatography on silica gel. The column was eluted with 1% MeOH/$CHCl_3$ and the eluate concentrated to yield 536 mg (78%) of (164) as an oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.28 (m, 2, H-1'a, H-1'b), 3.67 (dd, $J_{6-6'}$ = 11.0 Hz, J_{6-5} = 5.5 Hz, 1, H-6), 3.84-4.33 (m, 5), 4.4-4.85 (m, 9), 7.2-7.4 (m, 20, 4 x C_6H_5); MS m/z 573.1139 (3.6%,

$M^+-C_7H_7[C_{28}H_{30}O_5I] = 573.1139$), 359.0182 (2.6%, $M^+-2 \times C_7H_7O$), 91.0513 (100%, C_7H_7); Anal. ($C_{35}H_{37}IO_5$) Calc: C, 63.26; H, 5.61. Found: C, 63.27; H, 5.66.

Diethyl (2,3,5,6-tetra-O-benzyl-α-D-glucofuranosyl)-methylphosphonate (165)

A 450 mg (0.068 mmol) sample of (164) was refluxed under argon for 4 h in 5.6 mL of triethyl phosphite. After the excess of the reagent was removed in vacuo, the residue was chromatographed on silica gel. The product was eluted with 1% MeOH/$CHCl_3$ and the eluate concentrated to give 401 mg (88%) of (165) as an oil: $[\alpha]_D^{20} = -14°$ (c = 0.66, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.28 (m, J = 7.0 Hz, 6, 2 x $-CH_2CH_3$), 2.2 (m, 2, H-1'a, H-1'b) 3.68 (dd, $J_{6-6'} = 11.0$ Hz, $J_{6-5} = 6.0$ Hz, 1, H-6), 3.9 (dd, $J_{6'-6} = 11.0$ Hz, $J_{6'-5} = 2.0$ Hz, 1, H-6'), 3.97 (d, $J_{2-1} = 3.4$ Hz, 1, H-2), 4.02 (m, 1, H-5), 4.08 (m, 4, 2 x $-CH_2CH_3$), 4.12 (d, $J_{3-4} = 4.0$ Hz, 1, H-3), 4.22 (dd, $J_{4-3} = 4.0$ Hz, $J_{4-5} = 9.5$ Hz, 1, H-4) 4.45-4.84 (m, 8, 4 x $-CH_2Ph$), 4.54 (m, 1, H-1), 7.24-7.4 (m, 20, 4 x C_6H_5); ^{31}P NMR ($CDCl_3$, ext. ref. H_3PO_4) + 28.92 ppm; MS m/z 674.3009 (0.2%, $M^+[C_{39}H_{47}O_8P] = 674.3011$), 353.1486 (0.2%, $M^+-3 \times C_7H_7O$), 246.1022 (1.6%, $M^+-4 \times C_7H_7$), 91.0548 (100%, C_7H_7); Anal.

($C_{39}H_{47}O_8P$) Calc: C, 69.42; H, 7.02. Found: C, 69.22; H, 7.03.

Diethyl 3,6(R),7-tri-O-benzyl-5(S)-hydroxy-1,3 (E,E)-heptadienylphosphonate (167)

A 270 mg (0.5 mmol) sample of (47) in 750 μl of CH_2Cl_2 was stirred vigorously with 173 mg (0.6 mmol) of tetraethyl methylenebisphosphonate in 750 μl of 50% aq. NaOH for 24 h at r.t. The aqueous phase was separated and washed with 2 x 1 mL of CH_2Cl_2. The combined CH_2Cl_2 layer was washed with water, sat. NaCl/H_2O, dried over Na_2SO_4 and evaporated to give a residue which was chromatographed on silica gel. The product was eluted with 1% MeOH/$CHCl_3$ and the eluate concentrated to yield 109.4 mg (38%) of (167) as an oil: $[\alpha]_D^{20}$ = -22° (c = 0.46, $CHCl_3$); UV (MeOH) max 257 nm (ε 9,200), min 231 nm (ε 6,100); ^{1}H NMR (400 MHz) δ 1.2 ("t",* J = 7.0 Hz, 2 x $-CH_2\underline{CH_3}$), 3.54-3.7 (m, 3), 3.98 (m, J = 7.0 Hz, 2 x $-\underline{CH_2}CH_3$), 4.5-4.76 (m, 7), 5.2 (d, J_{OH-5} = 5.5 Hz, 1, OH), 5.67 (d, J_{4-5} = 9.5 Hz, 1, H-4) 5.86 (m, J_{2-1} = 17.0 Hz, 1, H-2), 6.88 (m, J_{1-2} = 17.0 Hz, 1, H-1), 7.23-7.4 (m, 15, 3 x C_6H_5); ^{31}P NMR (ext. ref. H_3PO_4) + 18.90 ppm; MS m/z (glycerol/FAB) =

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* "t" = overlapping triplets.

567 (MH^+); Anal. ($C_{32}H_{39}O_7P \cdot 0.25\ H_2O$) Calc: C, 67.30; H, 6.97. Found: C, 67.09; H, 6.85.

Reaction of (47) with tetraethyl methylenebisphosphonate/NaH

A 108 mg (0.2 mmol) sample of (47) in 600 μl of THF-HMPA (4:1) was treated at -23°C with 11.52 mg (0.48 mmol) of NaH (50% dispersion in oil was washed with benzene under an atmosphere of argon and transferred to the above solution in 600 μl of THF-HMPA [4:1]). A solution of 69.12 mg (0.23 mmol) of tetraethyl methylenebisphosphonate in 400 μl of THF-HMPA (4:1) was added to the above reaction mixture and stirring continued at -23°C for 30 min and then at room temperature for 4 h. After evaporation of the volatile solvents, the residue was taken up in ~10 mL of $CHCl_3$ and washed with H_2O, sat. $NaCl/H_2O$, dried over Na_2SO_4 and evaporated. The resulting product was chromatographed on a silica gel column using cyclohexane-acetone (3:1) as the eluant. Evaporation of appropriate fractions gave 101 mg (75%) of a mixture of (165) and (166): ^1H NMR ($CDCl_3$, 200 MHz) δ 1.28 (m, 6, 2 x $-CH_2\underline{CH_3}$), 2.1-2.3 (m, 2, H-1'a, H-1'b), 3.68 (m, 1, H-6), 3.85-4.2 (m, 8), 4.5-4.9 (m, 8), 7.2-7.4 (m, 20, 4 x C_6H_5); ^{31}P NMR ($CDCl_3$, ext. ref. H_3PO_4) +27.12 ppm

(β), 29.12 (α) ppm ratio 3:2; MS m/z 674.3000 (0.3%, $M^+[C_{39}H_{47}O_8P]$ = 674.3008), 583.2451 (0.4%, $M^+-C_7H_7$), 246.1022 (0.7%, $M^+-4 \times C_7H_7$), 91.0536 (100%, C_7H_7); MS m/z (glycerol/FAB) = 675 (MH^+).

Further elution of the column with cyclohexane-acetone (3:1) yielded 11 mg (10%) of (167): MS m/z (glycerol/FAB) = 567 (MH^+). The spectral properties of this compound were identical to the previously mentioned data.

(2,3,5,6-Tetra-O-benzyl-α-D-glucofuranosyl)methyl alcohol (172) and (2,3,5,6-Tetra-O-benzyl-β-D-glucofuranosyl)-methyl alcohol (173)

A 54 mg (0.1 mmol) sample of (162) in 5 mL of 1,2-dichloroethane was treated with 25.88 mg (0.16 mmol) of MCPBA and the reaction mixture refluxed for 20 h. It was then partitioned between CH_2Cl_2 and H_2O. The organic layer was washed successively with sat. aq. $NaHCO_3$, H_2O, sat. aq. NaCl, dried over Na_2SO_4 and evaporated to give an oil, which was chromatographed on silica gel. The column was eluted with 0.5% $MeOH/CHCl_3$. Evaporation of appropriate fractions gave 17 mg (31%) of (173) as an oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 2.03 (dd, $J_{OH-1'a}$ = 4.5 Hz, $J_{OH-1'b}$ = 4.0 Hz, 1, OH), 3.68-4.86 (m, 17), 7.18-7.42

(m, 20, 4 x C_6H_5); MS m/z 463.2120 (0.7%, M^+-C_7H_7) [$C_{28}H_{31}O_6$] = 463.2120), 372.1529 (0.05%, M^+-2 x C_7H_7), 91.0521 (100%, C_7H_7); Anal. ($C_{35}H_{38}O_6$) Calc: C, 75.79; H, 6.91. Found: C, 75.35; H, 6.88.

Further elution of the column with the same solvent mixture gave 18.2 mg (33%) of (172) as an oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 2.26 ("t",* $J_{OH-1'a}$ = 5.75 Hz, $J_{OH-1'b}$ = 5.0 Hz, 1, OH), 3.6-3.94 (m, 4), 4.0-4.2 (m, 4), 4.38-4.84 (m, 9), 7.2-7.44 (m, 20, 4 x C_6H_5); MS m/z 463.2123 (2.2%, M^+-C_7H_7[$C_{28}H_{31}O_6$] = 463.2120), 249.1140 (1.8%, M^+-2 x C_7H_7O-C_7H_7), 91.0519 (100%, C_7H_7); Anal. ($C_{35}H_{38}O_6$) Calc: C, 75.79; H, 6.91. Found: C, 75.40; H, 6.96.

Octanoyl Isocyanate (175)

A solution of 7 mL (6.67 g, 4 mmol) of octanoyl chloride in 40 mL of anhydrous Et_2O was added dropwise with stirring to a suspension of 8 g (5.3 mmol) of AgNCO in 20 mL of anhydrous Et_2O under argon. After the addition was complete, the reaction mixture was refluxed for 3 h and filtered to remove the precipitated AgCl. The filtrate was evaporated and the residue distilled at bp. 53-55°C/0.7 Torr to give 6.93 g (58%) of (175): MS m/z

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* "t" = overlapping triplets.

169.1094 ($M^+[C_9H_{15}NO_2]$ = 169.1102); Anal. ($C_9H_{15}NO_2$) Calc: C, 63.88; H, 8.93; N, 8.28. Found: C, 63.78; H, 8.98; N, 8.04.

6-N-Benzoyl-2',3'-O-isopropylidene-5'-O-[(N-octanoyl)-carbamoyl]adenosine (176)

A 411 mg (0.1 mmol) sample of 6-N-benzoyl-2',3'-O-isopropylideneadenosine (150) dissolved in 5 mL of dry CH_2Cl_2 was treated with 202 mg (0.12 mmol) of octanoyl isocyanate (175) at room temperature under rigorously anhydrous conditions. After 2 h, the reaction mixture was evaporated to dryness and the residue coevaporated with $CHCl_3$ to give 514 mg (88%) of (176): mp. 68-70°C; UV (MeOH) max 280 nm (ε 20,700); min 247 nm (ε 7,600); (H^+) max 287 nm (ε 24,000), min 243 nm (ε 11,700); (OH^-) max 302 nm (ε 13,800), min 259 nm (ε 9,300); ^1H NMR (200 MHz) δ 0.82 (t, J = 7.0 Hz, 3, $-CH_2\underline{CH_3}$), 1.1-1.3 (m, 10, 5 x CH_2), 1.35, 1.56 (2 s, 2 x 3, $-CMe_2$), 2.37 (t, 2, $-C(O)CH_2$), 4.2 (m, 2, H-5', H-5"), 4.5 (m, 1, H-4'), 5.1 (dd, $J_{3'-2'}$ = 6.0 Hz, $J_{3'-4'}$ = 2.5 Hz, 1, H-3'), 5.58 (dd, $J_{2'-1'}$ = 3.0 Hz, $J_{2'-3'}$ = 6.0 Hz, 1, H-2'), 6.33 (d, $J_{1'-2'}$ = 3.0 Hz, 1, H-1'), 7.7-8.1 (m, 5, C_6H_5), 8.63 (s, 1, H-2), 8.78 (s, 1, H-8), 10.52 (s, 1, 6-NH), 11.24 (s, 1, NH); MS m/z (glycerol/FAB) = 581 (MH^+); Anal.

($C_{29}H_{36}N_6O_7$) Calc: C, 59.99; H, 6.25; N, 14.47. Found: C, 59.95; H, 6.25; N, 14.83.

6-*N*-Benzoyl-5'-*O*-[(*N*-octanoyl)carbamoyl]adenosine (177)

A 29 mg (0.05 mmol) sample of (176) was stirred with 2 mL of 90% CF_3CO_2H/H_2O at room temperature for ~10 min. Evaporation of volatile solvents and recrystallization of the residue from $CHCl_3$ by diffusion of Et_2O gave 23 mg (96%) of (177): mp 100-102°C; UV (MeOH) max 280 nm (ε 20,700), min 226 nm (ε 12,900); (H^+) max 287 nm (ε 23,600), min 241 nm (ε 10,700); (OH^-) max 302 nm (ε 13,600), min 257 nm (ε 8,500); ^1H NMR (200 MHz) δ 0.85 (t, J = 7.0 Hz, 3, $-CH_2\underline{CH_3}$), 1.2-1.6 (m, 10, 5 x CH_2), 2.44 (t, J = 7.5 Hz, 2, $-CH_2\underline{CH_2}-CO-$), 4.15-4.45 (m, 4, H-3', H-4', H-5', H-5"), 4.82 (m, 1, H-2'), 5.5 (d, $J_{OH-3'}$ = 5.0 Hz, 1, OH-3'), 5.75 (d, $J_{OH-2'}$ = 5.9 Hz, 1, OH-2'), 6.1 (d, $J_{1'-2'}$ = 6.0 Hz, 1, H-1'), 7.5-8.1 (m, 5, C_6H_5), 8.72 (s, 1, H-2), 8.78 (s, 1, H-8), 10.63 (s, 1, 6-NH), 11.22 (s, 1, NH); MS m/z (glycerol/FAB) = 541 (MH^+); Anal. ($C_{26}H_{32}N_6O_7$) Calc: C, 57.77; H, 5.97; N, 15.55. Found: C, 57.49; H, 5.91; N, 15.17.

6-*N*-Benzoyl-9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (178)

A sample of 251 mg (1 mmol) of (3) was converted to the title compound using identical conditions as described for 2'-deoxyadenosine.[205] This gave 80 mg (23%) of (178): mp 94-96°C; UV (H_2O) max 280 nm (ε 20,800); (H^+) max 285 nm (ε 22,400); ^1H NMR (200 MHz) δ 2.08 (m, 1, H-3'), 2.28 (m, 1, H-3"), 3.64 (m, 2, H-5', H-5"), 4.13 (m, 1, H-4'), 4.62 (m, 1, H-2'), 5.18 (br, 1, OH-5'), 5.52 (br, 1, OH-2'), 6.32 (d, $J_{1'-2'}$ = 5.5 Hz, 1, H-1'), 7.4-7.7, 7.98-8.1 (m, 5, C_6H_5), 8.67 (s, 1, H-2), 8.74 (s, 1, H-8), 11.18 (br s, 1, NH); MS m/z 355.1284 (1.6%, $M^+[C_{17}H_{17}N_5O_4]$ = 355.1281), 338.1254 (1.8%, M^+-OH), 135.0547 (64.1%, M^+-COC_6H_5-sugar [$C_5H_5N_5$]), 164.0574 (100%, BHCHO-COC_6H_5).

6-*N*-Benzoyl-5'-*O*-[(*N*-octanoyl)carbamoyl]-9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (179)

A 20 mg (0.056 mmol) sample of (178) in 30 mL of dry CH_2Cl_2-dioxane-CH_3CN (1:1:1) was treated with 12 μl (0.07 mmol) of octanoyl isocyanate (175) and the reaction mixture stirred at room temperature under a strong flow of argon for 4 h. An additional 6 μl (0.035 mmol) of (175) was added and stirring continued for 2 h. Evaporation and chromatography of the residue by PLC using 10% MeOH/$CHCl_3$ yielded 17 mg (58%) of (179): mp 92-95°C; UV (MeOH) max 280 nm, min 248 nm; (H^+) max 288 nm, min 262 nm; (OH^-) max 303 nm, min 261 nm; ^1H NMR (200 MHz) δ 0.83 (t, J = 7.0 Hz, 3, $-CH_2\underline{CH_3}$), 1.2-1.48 (m, 10, 5 x CH_2), 2.09 (m, 1, H-3'), 2.32-2.54 (m, 3, H-3", $-C(O)CH_2$), 4.36 (m, 3, H-4', H-5', H-5"), 4.61 (m, 1, H-2'), 5.68 (br, 1, OH-2'), 6.36 (d, $J_{1'-2'}$ = 5.5 Hz, 1, H-1'), 7.46-7.7, 8-8.1 (m, 5, C_6H_5), 8.53 (s, 1, H-2), 8.72 (s, 1, H-8), 11.7 (br, 2, 2 x NH); MS m/z (glycerol/FAB) = 525 (MH^+).

5'-Amino-5'-deoxy-6-*N*-formyl-2',3'-*O*-isopropylideneadenosine (180)

A 180 mg (0.05 mmol) sample of (112) was hydrogenolyzed over 115 mg of 5% Pd-C catalyst in 27 mL of EtOH/H_2O (20:7) at 35 psi H_2 for 20 h at room temperature in a Parr shaking apparatus. Filtration of the mixture with a celite pad and evaporation of solvent gave a residue which was chromatographed on silica gel using 20% MeOH/$CHCl_3$ to give 70 mg (43%) of (180): mp 88-90°C; UV (MeOH) max 272 nm, min 232 nm; ^1H NMR (200 MHz) δ 1.28, 1.5 (2 s, 2 x 3, $-CMe_2$), 3.33 (m, 2, H-5', H-5"), 4.18 (m, 1, H-4'), 4.92 (dd, $J_{3'-2'}$ = 3.0 Hz, $J_{3'-4'}$ = 3.5 Hz, 1, H-3'), 5.4 (dd, $J_{2'-3'}$ = 3.0 Hz, $J_{2'-1'}$ = 2.5 Hz, 1, H-2'), 6.13 (d, $J_{1'-2'}$ = 2.5 Hz, 1, H-1'), 7.38 (s, 1, NH_2), 8.05 (d, 1, H-2), 8.18 (s, 1, H-8), 8.34 ("d", 2, NH, CHO); MS m/z 334.1388 (0.5%, $M^+[C_{14}H_{18}N_6O_4]$ = 334.1378), 164.0571 (100%, B + 2H), 135.0548 (44.8%, M^+-CHO-sugar $[C_5H_5N_5]$). Anal. ($C_{14}H_{18}N_6O_4 \cdot 0.5\ H_2O$) Calc: C, 48.98; H, 5.58; N, 24.48. Found: C, 48.74; H, 5.29; N, 24.47. Further elution of the column with 20% MeOH/$CHCl_3$ gave 64 mg (42%) of (181) after recrystallization from EtOAc-petroleum ether (60-90°): mp 203-206°C; the spectral properties of this compound were identical with published data.[149]

5'-Deoxy-5'-N[(N-octanoyl)carbamoyl]amino-6-N-formyl-2',3'-O-isopropylideneadenosine (182)

A 80 mg (0.24 mmol) sample of (180) in 2.5 mL of dry CH_2Cl_2 was treated with 70 μl (0.41 mmol) of octanoyl isocyanate (175) and the reaction mixture stirred at room temperature under a strong flow of argon for 2 h. Evaporation of volatile solvents gave a residue which was chromatographed on silica gel using 1% MeOH/$CHCl_3$. Evaporation of appropriate fractions yielded 102 mg (85%) of (182): mp 84-86°C; UV (MeOH) max 272 nm (ε 19,800), min 231 nm (ε 2,100); (H^+) max 274 nm (ε 19,600), min 236 nm (ε 4,000); (OH^-) max 298 nm (ε 19,500), min 240 nm (ε 5,000); ^1H NMR (300 MHz) δ 0.84 (t, J = 7.0 Hz, 3, $-CH_2\underline{CH_3}$), 1.2-1.4 (m, 16, 5 x CH_2, $-CMe_2$), 2.45 (t, 1, $-C(O)CH_2$), 3.42 (m, 2, H-5', H-5"), 4.2 (m, 1, H-4'), 4.98 (dd, $J_{3'-2'}$ = 3.0 Hz, $J_{3'-4'}$ = 3.2 Hz, 1, H-3'), 5.5 (dd, $J_{2'-1'}$ = 2.5 Hz, $J_{2'-3'}$ = 3.0 Hz, 1, H-2'), 6.27 (d, $J_{1'-2'}$ = 2.5 Hz, 1, H-1'), 8.05 (d, 1, CHO), 8.22 (t, 1, $CH_2\underline{NH}$), 8.66 (s, 1, H-2), 8.7 (s, 1, H-8), 11.14 (br, 1, 6-NH), 11.75 (s, 1, NH); MS m/z (glycerol/FAB) = 504 (MH^+); Anal. ($C_{23}H_{33}N_7O_6$) Calc: C, 54.86; H, 6.61; N, 19.47. Found: C, 54.58; H, 6.60; N, 19.40.

5'-Deoxy-5'-*N*-[(*N*-octanoyl)carbamoyl]amino-6-*N*-formyladenosine (183)

A 50 mg (0.1 mmol) sample of (182) was stirred in 2 mL of 90% CF_3CO_2H/H_2O for 5 min at room temperature. Evaporation of volatile solvents *in vacuo* gave a residue, which upon recrystallization from $CHCl_3$ by diffusion of Et_2O gave 45 mg (97%) of (183): mp 105-106°C; UV (MeOH) max 272 nm (ε 19,900), min 231 nm (ε 2,100); (H^+) max 275 nm (ε 19,700), min 237 nm (ε 4,500); (OH^-) max 298 nm (ε 19,500), min 242 nm (ε 5,100); ^1H NMR (300 MHz) δ 0.82 (t, J = 7.0 Hz, 3, $-CH_2\underline{CH_3}$), 1.2-1.7 (m, 10, 5 x CH_2), 2.45 (t, 2, $-C(O)CH_2$), 3.32-3.6 (m, 3, H-5', H-5", OH), 3.96 (m, 1, H-4'), 4.11 (m, 1, H-3'), 4.76 (m, 1, H-2'), 5.5 (br, 1, OH), 6.0 (d, $J_{1'-2'}$ = 6.0 Hz, 1, H-1'), 8.08 (d, 1, CHO), 8.25 (t, 1, $CH_2N\underline{H}$), 8.67 (s, 1, H-2), 8.72 (s, 1, H-8), 11.14 (br, 1, 6-NH), 11.7 (s, 1, NH); MS m/z (glycerol/FAB) = 464 (MH^+); Anal. ($C_{20}H_{29}N_7O_6$) Calc: C, 51.83; H, 6.31; N, 21.15. Found: C, 51.73; H, 6.33; N, 21.47.

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